

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



	TABLE OF CONTENTS	
	TABLE OF CONTENTS	
IIA 8	Ecotoxicological Studies on the Active Substance  Avian toxicity  Acute oral toxicity to quail species, mallard duck or other bird  Avian dietary toxicity (5-day) test in quail species or mallard duck  Avian dietary toxicity (5-day) test in a second unrelated species  Subchronic and reproductive toxicity to birds	**Page   6
IIA 8.1	Avian toxicity	<b>4</b> 66
IIA 8.1.1	Acute oral toxicity to quail species, mallard duck or other bird	§ 7 <u>%</u>
IIA 8.1.2	Avian dietary toxicity (5-day) test in quail species or mallard duck	7 7 % <b>17</b>
IIA 8.1.3	Avian dietary toxicity (5-day) test in a second unrelated species	<b>26</b>
IIA 8.1.4	Subchronic and reproductive toxicity to birds	<b>26°</b>
IIA 8.2	Fish toxicity	<i>©′</i> <b>₹44</b>
IIA 8.2.1	Acute toxicity of the active substance to fish	46
IIA 8.2.1.1	Subchronic and reproductive toxicity to birds  Fish toxicity  Acute toxicity of the active substance to fish  Rainbow trout (Oncorhynchus mykiss)  Effects on amphibians  Warm water fish species	46
	Effects on amphibians	49
IIA 8.2.1.2	Warm water fish species & & & & & & & & & & & & & & & & & & &	52
IIA 8.2.1.3	Acute toxicity of metabolities to the more sensitive of fish species	60
IIA 8.2.2	Chronic toxicity to fish  Chronic foxicity (28 day exposure) to juvenile fish  Fish early life stage toxicity test	67
IIA 8.2.3	Chronic foxicity (28 day exposure) to juyenile fish @	68
IIA 8.2.4	Fish early life stage toxicity test	68
IIA 8.2.5	Fish life cycle test	72
IIA 8.2.6	Bioconcentration potential in fish	<b>72</b>
IIA 8.2.6.1	Bioconcentration potential of the active substance in fish	<b>72</b>
IIA 8.2.6.2	Bioconcentration potential of the metabolites, degr. & react. products	72
IIA 8.2.7	Aquatic bigavailability/biomagnification / depuration	72
IIA 8.3	Toxicity to aquatic species other than fish, aquatic field tests	72
IIA 8.3.1	Acute toxicity to aquatic invertebrates	73
IIA 8.3.1.1	Acute toxicity (24 and 48 hour) for Daphnia preferably (Daphnia magi	na)73
IIA 8.3.1.2	Acute toxicity (24/98 h) for representative species of aquatic insects	<b>79</b>
IIA 8.3.1.3	Acute toxicity for representative species of aquatic crustaceans	90
IIA 8.3.1.4	Scute toxicity for repr. species of aquatic gastropod molluscs	90
IIA 8.3.2	Chapnic toxicity to aquatic invertebrates	91
IIA 8.3.2	Chronic toxicity in Daphnia magna (21-day)	91
IIA 8.3.2.2	Chronic toxicity for representative species of aquatic insects	98
IIA 8.3.2.3	Chronic toxicity for repr. species of aquatic gastropod molluscs	112

IIA 8.3.3	Aquatic field testing	112
IIA 8.4	Effects on algal growth and growth rate (2 species)	113
IIA 8.5	Effects on sediment dwelling organisms	<b>326</b>
IIA 8.5.1	Acute test	126
IIA 8.5.2	Chronic test	<b>127</b>
<b>IIA 8.6</b>	Chronic test  Effects on aquatic plants  Effects on bees  Acute oral toxicity	<sup>©</sup> 12 <b>7</b>
IIA 8.7	Effects on bees	132
IIA 8.7.1	Acute oral toxicity	9 132 J
IIA 8.7.2	Acute contact toxicity	165
IIA 8.7.3	Acute contact toxicity  Toxicity of residues on foliage to honey bees  Bee broad feeding test	<sup>2</sup> 165
IIA 8.7.4	Bee brood feeding test	165°
IIA 8.8	Effects on non-target terrestrial arthropods	<b>165</b>
IIA 8.8.1	Effects on non-target terrestrial arthropods artificial substrates  Parasitoid  Predatory mites	165
IIA 8.8.1.1	Parasitoid Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q	166
IIA 8.8.1.2	Predatory mites & & & & & & & & & & & & & & & & & & &	169
IIA 8.8.1.3	Ground dwelling predatory species	172
IIA 8.8.1.4	Foliage dwelling predatory species	172
IIA 8.8.2	Effects on non-target terrestrial arthropods in extended lab/semi-fiel	d test
TT A O O O O		172
IIA 8.8.2.1	Barasitoid * * * * * * * * * * * * * * * * * * *	172
IIA 8.8.2.2	Predatory mites	172
IIA 8.8.2.3	Ground dwelling predatory species	172
IIA 8.8.2.4	Foliage dwelling predatory species.	172
IIA 8.8.2.5	Other terrestrial invertebrates  Effects on earthworms  Acute toxicity to earthworms	172
	Effects on earthworms  Acute toxicity to earthworms	172
IIA 8.9.1		173
IIA 8.9.2	Subjethal effects on earthworms	180
IIA <b>8</b> Ã0	Effects of soil microbal activity  Nitrogen transformation	190
IIA 8.10.1 IIA 8.10.2		190
¥ .	Carbon pineralization  Rates of recovery following treatment	195
IIA 8.10 \$\infty \text{IA 8.10}\$	Effects on marine and estuarine organisms	198 198
IIA 8.11,4	Marine or estuarine organisms acute toxicity LC50/EC50	198
IIA 8.11.2	Marine/Estuarine fish - salinity challenge	214
IIA 0.11.2	Effects on terrestrial vascular plants	214

Tier 2, IIA, Se	c. 6, Point 8: Flupyradifurone (BYI 02960)	
IIA 8.13	Effects on terr. vertebrates other than birds / wild mammal toxicity	221
IIA 8.14	Effects on other non-target organisms believed to be at risk	<b>221</b>
IIA 8.14.1	Summary of preliminary data: biological activity & dose range finding	g239
IIA 8.14.2	Assessment of valovance to notantial impact on non-target species	2) 2/12
IIA 8.15	Effects on biological methods for sewage treatment	<b>2</b> 43
IIA 8.16	Other/special studies	<sup>2</sup> 24 <b>7</b>
IIA 8.16.1	Effects on biological methods for sewage treatment Other/special studies Other/special studies - laboratory studies Effects on honey bees	<b>2</b> 47
	Effects on honey bees	<b>247</b>
IIA 8.16.2	Other/special studies - field studies	283
	Effects on honey bees  Other/special studies - field studies  Effect of BYI 02960 on soil litter bag degradation  Summary and evaluation of points IIA 7 and IIA 8.1 to 8.16  Summary of Point IIA 7, Environmental Fate  Summary of IIA point 8  List of BYI 02960 metabolites included in this section	283
IIA 8.17	Summary of Point HA 7 Extrapolate Page 11A 8.1 to 8.16	' <b>2</b> 93°
	Summary of Point II 4.7, Environmental Fate  Summary of IIA point 8  List of BYI 02960 metabolites included in this section	<b>2</b> 93
	Summary of IIA point 84 2 2 2 2	294
	List of BYI 02960 metabolites included in this section	300
4		
, W		
~		
Ş		
Ţ		



### **IIA 8 Ecotoxicological Studies on the Active Substance**

### **IIA 8.1** Avian toxicity

Avian toxicity testing was performed following the requirements in various regulatory regions and countries.

The lowest acute oral LD<sub>50</sub> of 232 mg a.i. /kg b.w. was observed in the standard testing species Northern Bobwhite quail (Colinus virginianus). Additional acute oral toxicity testing in the Canary (Serinus canaria) and the hen (Gallus gallus domesticus) resulted in higher LD<sub>50</sub> values.

Short term dietary toxicity studies were conducted with chicks of the Northern Bolowhite quail and of the Mallard duck (Anas platyrhynchos). In these studies, no mortality occurred in to the nominal concentration of 5000 ppm, corresponding to an achieved daily dietary doses of \$25 mg a is by b. Wid in the ducklings and 470 mg a.i. /kg b.w./d in the quail chicks. «

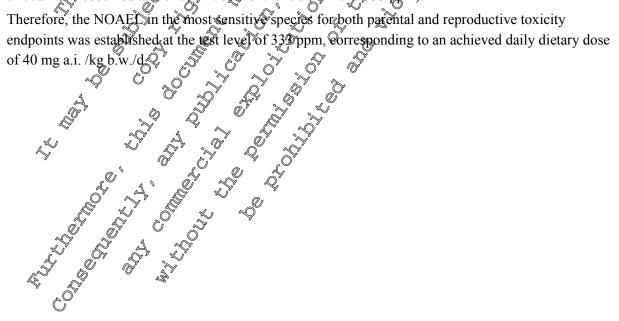
Based on a significant reduction of the 8-d bodyweight at 2075 ppon (262 mg a j/kg b.0./d), the Bobwhite quail was considered the more sensitive species also in short term detary testing. It is worth noting, that the toxicity of BYI 02960 from respected dietary exposure of that most sensitive species was found to be much less pronounced than toxicity from single acute oral tosing

Reproduction toxicity testing was conducted in the most sensitive species (Northern Bobwhite quail) and in the Mallard duck.

In the Mallard duck, the NOAELA for both parental and reproductive toxicity endpoints was found at the top test level of 845 ppin (achieved daily dietary dose 81 mg a.i./log b.w./d).

Exposure of Northern Bobyshite Quail to BYI 02960 prior to and during their reproduction resulted in clear effects on parental survival, body weight and health at the top dose level of 1000 ppm. The number of eggs aid by the birds at this top dose level was eignificantly reduced, resulting in a reduced number of hatchlings and 14-d survivors. No treatment related, statistically and biologically relevant effects were observed at the lower treatment levels (201 and 333 ppm).

Therefore, the NOAED in the most consitive species for both parental and reproductive toxicity





# IIA 8.1.1 Acute oral toxicity to quail species, mallard duck or other bird

Report:	KIIA 8.1.1/01; , T.B. &	, T.L. (2010)
Title:	Toxicity of BYI 2960 Technical Durin	g an Acute Oral LD50 worth the Norther
	Bobwhite Quail ( <i>Colinus virginianus</i> )	
Report No:	EBRVP022	
Document No:	M-386036-01-1	
Guidelines:	OPPTS 850.2100	
	OECD Guideline 223	
<b>Deviations:</b>	None	
GLP:	Yes (certified laboratory)	
	Some data (screening of feed and corn-	oil for contaminants) were not performed
	according to GLP, as described in the s	study report

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the acute effects of BYI 02960 (Sample description: TOX 08508-00 (Batch ID: 2009-000239), purity 96.2% w/w) to Northern Bobwhite Quait (Colinus virginianus).

Nineteen-week old adults were orally dosed via gelatine capsules at 0.25, 500 100, 200 and 400 mg a.i./kg body weight, respectively, and subsequently monitored for a period of 14 days. Mortality, signs of intoxication, food consumption body weight and gross necropsy results were evaluated to determine the endpoints.

There were no mortalities in the control, 25, 50 and 500 mg A.i./kg b.w. treatment groups. Mortality of 40 and 90% was observed at 200 and 400 mg a.i./kg b.w. respectively.

No clinical signs of oxicity were observed in the control, 25 or 50 mg a.i./kg b.w. treatment groups. Hypo-reactivity to stimuli was observed in the 100,200 and 400 mg a.i./kg b.w. treatment groups. Postmortem examinations were generally unremarkable, with the exception that several birds were found with fluid in their gastrointestinal tracts.

Body weight on DAT was significantly reduced of the 200 mg a.i./kg b.w. treatment level compared to the control birds. The NOAEL was considered to be 300 mg a.i./kg b.w.

The acute oral LD<sub>50</sub> for BYI 02960 technical in northern bot white quail was 232 mg a.i./kg body weight (95% CF = 176 to 315 mg a.i./kg body weight).

### MATERIAL AND METHODS

### A. Materials

### 1. Test material

Test item: BYI 02960

Type of test material: Substance, technical Chemical state and Beige powder

description:

2009-000239 Batch number: Sample description: TOX 08508-00

2(5H)-Furanone, 4-[[(6-chloro-3-pyridiny)methyl](2,2 CAS name:

difuoroethyl)amino]

CAS#: 951659-40-8

4-[(6-chloropyridin@ **IUPAC** name:

96.2% w/w Purity:

Stability of test compound: Expiry date:

### 2. Test organisms

Species: Colinus Arginiann Northern Bobwhite Quai Common name:

Source:

Age at study initiation:

Maintenance prior to test:

Period of acclimation to test conditions:

# B. Study design and methods

1. In life dates:

2. Experimental treatments

Colinus virginianus (19) weeks old) were administered orally with BYI 92960; (purity 96.2% w/w). After the birds had received 25, 50, 100, 200 and 400 mg a judg body weight via gelatine capsule the birds were observed over a period of 14 days. Each age served a sone treatment level containing 5 (males and females separately) birds

### 3. Observation and measurements

Mortality and rights of intoxications were assessed Body weight measurements were conducted at days -1, 7 and 14.3 The food consumption was calculated from weighing the residual food at 0 - 7,

7 - 14 and 9 - 14 days after treatment. Gross recropsies were conducted after test termination.

Mortality data were analyzed will a multi-method program (CT-Tox) that can determine the LC<sub>50</sub> and 95% confidence interval using non-linear interpolation, Binomial, Moving Average, Probit, and Spearman-Karber methods

For body Weight and growth, normality and homogeneity of variance of the data were tested using the Shapiro Wilk's est ( $\alpha = 0.01$ ) and the Levene's test ( $\alpha = 0.05$ ), respectively. Normally distributed data were Subjected to standard one-way ANOVA followed by Dunnett's test or Bonferroni t-test.

### RESULTS AND DISCUSSION

### A. Environmental Conditions

Birds were kept under conditions which are summarized as follows:

Mean 21°C Test temperature: Mean 54% Relative humidity:

10 hours light / 14 hours dark Photoperiod:

Light source Natural daylight

15 changes per hour (average) Air change:

els of 25,50 B. Biological Findings

Bird mortality: No mortalities occurred during the study in the control and at the dose levels of 25. and 100 mg a.i./kg b.w., respectively. At 200 and 400 mg a.i.kg b.w. respectively. 40 and 90% mortality occurred. The LD<sub>50</sub> of 232 mg a.i./kg b.w/95% L: 1720 to 313 mg a i./kg bw/, slope 5.9 (95% CL 2.5-9.3), was estimated by probit analysis.

Body weight and food consumption: Body weight data were normally distributed and variances were homogeneous; therefore, parametric statistical procedures were conducted with Bonterroni dest  $(\alpha = 0.05)$ . Male and female body weights in the 200 mga.i./kgb.w. level were significantly lower as compared to the control for Day.

Based on empirical analyses, there was a reduction in female feed consumption among treatment groups compared to the control group. However the male feed consumption among the treatment groups was greater than the control group throughout the study period.

Clinical observations No symptoms of toxicity were observed within the control, 25 or 50 mg a.i./kg body weight treatment groups, respectively. All birds appeared normal following dosing with no effects of regurgitation observed. Hypo-reactivity to timuli was observed in the 100, 200 and 400 mg a.i./kg body weight treatment groups.

Post-Morten examinations: Necropsy we aled no pathomorphological changes with the exception that

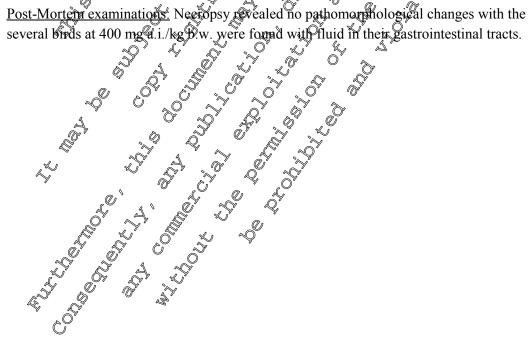


Table: Effect of BYI 02960 on mortality, body weight and food consumption of Colinus virginianus

Treatment level [mg a.i./kg	Sex	% mortality Day 14	Mean body weight [g]			Mean daily feed consumption [g/bird/day]	
b.w.]		Day 14	Day 0	Day 7	Day 14	[g/blid/day]	
untreated	male	0	$266.6 \pm 7.5$	$224.6 \pm 8.6$	$228.2 \pm 9.7$	$20.6 \pm 4.6$	
control	female	0	$225.2 \pm 5.4$	$222.6 \pm 5.3$	$225.2 \pm 5.5$	$27.8 \pm 10.2$	
25	male	0	$225.8 \pm 7.9$	$223.2 \pm 6.3$	$229.8 \pm 7.6$	25.3 ± 803	
23	female	0	$225.6 \pm 5.3$	220.6 ± 🗗	225.8 ± 19.9	22.5 7.4	(
50	male	0	$226.0 \pm 5.8$	222.8 ± 7.6	228.0, 8.0	$2300 \pm 5.9$	
30	female	0	$225.2 \pm 6.1$	2158 ± 4.2	$22306 \pm 8.4$	19.9 ± 5.6	
100	male	0	$226.8 \pm 6.1$	$223.8 \pm 9.6$	$228.8 \pm 99$	26.5 13.8	
100	female	0	$225.8 \pm 5.3$	$216.8 \pm 4.2$	220.8 ± 5.5	18.8 ± 7.1	
200	male	40	$226.6 \pm 6.4$	/ 209 <b>J</b> 4.6 💍	219,7±1.5	$29.9 \pm 17.5$	
200	female	40	$224.4 \pm 5.9$	$206.7 \pm 4.6^{h}$	296.0 ± 07	19.2 11.7	~ _&_°
400	male	100	227.2		A. (	n.d.	
400	female	80	224 6 ± 6.0	184 b	197 b	\$6.6 ± \$7	

<sup>&</sup>lt;sup>a</sup> Statistically different from the control group

# C. Validity Criteria

The validity criterion of control mortality

D. Biological Endpoints Derived Derived Derived Derived Derived:

LD<sub>50</sub>:

(95% Confidence limits: 173 to 313 mg a.i./kg bw)

The acute oral LaD<sub>50</sub> for BYI 02960 technication Noothern Bobwhite quail (Colinus virginianus) was 232 mg a.i./kg b.w. (95%ov) =

Report:	7
Titley	Exicity of BYI 2960 Schnical During an Acute Oral LD <sub>50</sub> with the Canary
	(Serinus canagia)
Report No: 🎸 🔬	EBRAPO36 V
Document No.	M&08514-01-1 @
Guidelines	OPPT\$ 850.2100
Guidelines	OECD Guideline 223
Deviations:	None
GLRY O	Yes (certified laboratory)
	Some data (contaminant screening of bird feed, determination of sex of canaries,
į į	screening of corn-oil and tap water) was not collected in accordance with GLP, the
	details are given in the study report

<sup>&</sup>lt;sup>a</sup> Statistically different from the control group be excluded from statistical comparisons that to sample size (n=1) n.d. not determined

## **EXECUTIVE SUMMARY**

The aim of the study was to determine the acute effects of BYI 02960 (Sample description: TOX 08508-00 (Batch ID: 2009-000239); purity 96.2% w/w) to Canary (Serinus canaria).

Adult Serinus canaria were orally dosed via gelatine capsules at 0, 44, 88, 175, 350 and 700 mg i./kg body weight, and subsequently monitored for a period of 14 days. Mortality, signs of intoxication, food consumption, body weight and gross necropsy results were evaluated to determine the endroints. There were no mortalities in the control, 44 and 88 mg a.i./kg b.w. treatment groups. Mortality in the 175, 350 and 700 mg a.i./kg b.w. treatment groups amounted to 50, 50 and 70%, respectively. Ataxia (loss of muscular coordination), hypo-reactivity to stimuli, and immobility were observed in the 44, 88, 175, 350 and 700 mg a.i./kg b.w. treatment groups. Severity and prevalence of chaical observations were primarily dose dependent, however several boods at each treatment level had minimal to no observed adverse effects. By 36 hours after dosing all bards recovered from observed symptoms of died. Body weight and food consumption were not significantly of ferent from the control treatment. ermined to be 330 mg a.i./kg b.w. @5% confidence limits: 215 to

ODS

BYI 02960

Substance, technical

1: Beige powder

2009-040239

TOX 08508-00

2(5N)-Furanone, 440 (6-chloro-3-pyridinyl)methyl](2,2-di@ioroethyl)amino]

951659-40-8 The acute oral LD<sub>50</sub> was determined to be 330 mg a.i.

# MATERIAL AND METHODS

### A. Materials

1. Test material

625 mg a.i./kg b.w.).

Test item: Type of test material

Chemical state and Description: Batch number: Sample description:

CAS name: 🧞

4-[(6ghloropyridin@-ylmethyl)(2,2-difluoroethyl)amino]furan-2(5H)-

ethy
1659-40-8
4-[(6conlorop
one)
96.2% w/w
Ambiem tempes
Gelatime capside
Detonised water Ambiem temposture

### 2. Test organisms

Species: Serinus canaria

Drinking water:
Period of acclimation to test conditions:
Starvation period;

B. Study design and methods
1. In life dates

August 31 to September 14, 2010

2. Experimental treatments

Serimus canaria were administered or ally with BYI 02960; (purity 96.2% w/w). After the birds had received 44, 88, 175, 350 and 700 mg a.i. kg b. w. a/ia gelatine capsule die birds were observed over a period of 14 days. In addition, dejonised water was tested as a regative control. Eact contained to the start of the start of

-1, 7 and 14. The food consumption was calculated from weighing the residual food at 1-7 days and 7 − 14 days after treatment. necropsies were conducted after test termination.

## 4. Statistical analysis

Mortality data were applyzed with a Sultimethod program (CT-Tox) that can determine the LC50 and 95% confidence interval using non-linear Interpolation, Binomial, Moving Average, Probit, and Spearman Karber methods.

For body weight and prowth 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. Normally distributed data were subjected to standard one way ANOV to followed by Dunnett's test or Bonferroni t-test.

Tier 2, IIA, Sec. 6, Point 8: Flupyradifurone (BYI 02960)

### RESULTS AND DISCUSSION

# A. Environmental Conditions

Birds were kept under conditions which are summarized as follows:

Test temperature: 24 °C (mean) Relative humidity: 48% (mean)

Photoperiod: 10 hours light/ 14 hours dark

Light intensity: 267 lux

Air changes: 16 changes per hour (Werage)

# B. Biological Findings

Dose-dependent mortality was observed at 175 mg a.i./kg b.w. and above. The acute oral Live was 330 mg a.i./kg body weight (95% CL = 215 to 625 mg a.i./kg body weight) The slope of the dose-response curve was 2.3 (95% CL = 1.1 to 3.5).

No statistically significant effects were observed on food consumption and body weight

Table: Effect of BYI 02960 promortality, food consumption and body weight of Springs Conaria

Treatment level [mg a.i./kg bw]	Sex	Mortality day 14	Body w	right (g) (mean	day 124	Daily feed Consumption (mean ± SD) [g/bird/day]
untreated	male	≫ Q4	21.2 1.3	y 21.3 <b>(¥</b> 1.1	2144±1.65	$3.4 \pm 0.4$
control	female 🛚 🖈		19.9±1.1	20,2 ± 1,3	$20.8 \pm 2.0$	$3.8 \pm 0.4$
44	male female		$20.8 \pm 20^{\circ}$	24.8 ± 1.9	$\bigcirc 20.7 \pm \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ $	$3.8 \pm 0.7$
	female	& 0, O)	$19.5 \pm 1.2$	∂ <sup>™</sup> 19.7±¶.1	19.6 ± 2.5	$3.1 \pm 0.3$
88	male	V Q'	20.7 ₹ 1.9	<sup>2</sup> 20.8± 1.6 ©		$3.2 \pm 0.7$
00	f@nale (		1905 ± 1.00	$20.7 \pm 1.6$	$20.6 \pm 1.1$	$3.6 \pm 1.0$
175	male &	<b></b> 40 €	20.8 ± 1.8	20.1 ± 0.9	$\sqrt{20.7 \pm 1.8}$	$3.5 \pm 0.4$
1/3	female	60,0	19.4	©(20.6 <sub>6</sub> 2,1.9) b <sub>©</sub>	(20.9–21.9) b	$(3.0-3.7)^{b}$
250	mare		$21.0 \pm 1.7$	(20.7-22.0)	(20.7–23.6) b	$(3.0 - 3.8)^{b}$
350	female	/ %40 *	10.6 ± 1.20	19.4 ± 0.9	$19.1 \pm 1.5$	$3.1 \pm 0.1$
700	maleू🏈 "	∜ 80 <sub>€</sub>	~ Qi.1 ±4.8	× 19.2	19.6 b	3.8a
/00	female		√″19.& <b>#</b> 1.1	(19.4 <del>)</del> 20.3) <sup>b</sup>	(19.6–20.5) b	$(2.6-4.1)^{b}$

<sup>&</sup>lt;sup>a</sup> SD = standard deviation

Clinical observations. Severity of clinical observations was primarily dose dependent, however several birds at each treatment level had minimal to no observed adverse effects. On day 0, at 44 mg a.i./kg bw and 88 mg a.i./kg bw. Tour birds were observed with a loss of muscular coordination (ataxia - still able to balance on percha, one and two birds, respectively, showed hyporeactivity and one and three birds, respectively, were immobile. All birds recovered on the same day. At 175 mg a.i./kg bw and 350 mg a.i./kg bw, all birds bowed symptoms of toxicity, five died and five recovered within < 31 hours after dosing. At 700 mg a.i./kg bw, seven birds died and the remaining three recovered from toxicity somptoms (ataxia, immobility) with 24 hours

b Range reported when only 2 surviving birds present. Value reported when only 1 surviving bird present

### C. Validity Criteria

The validity criterio	on of control mortality less than 10% was fulfilled.
<ul><li>D. Biological Endp</li></ul>	oints Derived
From the results pr	esented above the following biological endpoints can be derived:
LD <sub>50</sub> :	330 mg a.i./kg body weight (95% confidence limits:215 to 625 mg a.i./kg
	body weight)
Lowest lethal dose	(LLD): 175 mg a.i./kg b.w.
CONCLUSION	
JONCLOSION	
The acute oral LD <sub>5</sub>	o for BYI 02960 technical in Carary (Serinus Canaria) was 330 mg a.i./kg b.w.
95% CL = $215$ to	625 mg a.i./kg b.w.).
Report:	
	KIIA 8.1.1/932
Title:	KIIA 8.1.1/1932 , (2011) Acute oral rexicity of chicken (Gallus gallus domesticus) with BY 12960 (sech.)
Title:	KIIA 8.1.1/1934  Acute oral rexicity of chicken (Gallus gallus domesticus) with BYIS 2960 (tech.), according to OECAD 223 Limit text -
Title:  Report No:	KIIA 8.1.1/93; Acute oral rexicity of chicken (Gallus gallus domeorcus) with BYJ 2960 (tech.), according to OEQD 223 (Limit text -
Report No: Document No:	coints Derived esented above the following biological endpoints can be derived:  330 mg a.i./kg body weight (95% confidence limits:215 to 625 mg a.i./kg body weight)  (LLD): 175 mg a.i./kg b.w.  625 mg a.i./kg b.w.).  KIIA 8.1.1/03.  Acute oral toxicity of chicken (Gallus gallus domes reus) with BYE 2960 (tech.), according to DECD 223 2 Limit test -  BAR/LØ 141.  M-420519-01-2
Report No: Document No: Guidelines:	OECD Guideline 23
Document No.	171-42/9319-01-2
Guidelines:	OECD Guideline 23

The aim of the study was to determine the acute effects of BYI 02960 (Sample description: TOX 08508-00 (Batch ID: 2009-000239); purity 96.2% w/w to hears (Gallus domesticus) in a limit test, and was conducted in order to satisfy national data requirements in individual countries.

Five adult hens (treatment group) were orally administered with a single dose of 2000 mg a.i./kg bw via gelatine capsules and subsequently montrored for a period of 8 days. In addition, 10 control birds were kept under the same conditions from the beginning of the test until day 21 of the study (only 5 control birds from day 21 to day 280

Mortality, signs of intoxication, bood consumption, body weight and gross necropsy results were evaluated to determine the endpoints. Body weight were recorded prior to test initiation (day -1), on study day 3, 7, day 14, 21 and at test termination (day 28). Food consumption was measured daily until day 3, then for the periods 3-7 and 7-14, 14-21, and 21-28.

The most noticeable effect after the application was an almost complete reduction of food consumption with all dosed birds. All further observed symptoms were consequences of this starvation: excretion of uric acid Auffed Ceather or reduced vigilance. One bird started to feed in the 2<sup>nd</sup> week after application, another one in the 3rd week after application. Both birds showed soft excrements or diarrhoea as indication of digestion discress. A third bird behaved similarly in the 4th week after application.

Two dosed birds did not resume feeding at all after application of the test substance. They were sacrificed for humane reasons on day 28. Since all other birds were free of symptoms or showed clear indications of recovery by that time point, the test was terminated on day 28. Due to the reduced food

consumption all dosed birds showed severe body weight losses. Two birds started to regain body

weight in the last week of the test. The acute oral LD<sub>50</sub> was determined to be  $\geq$  2000 mg a.i./kg body weight...

### MATERIAL AND METHODS

### A. Materials

### 1. Test material

Test item: BYI 02960 Type of test material: Substance, technical Chemical state and description: Beige powder Batch number: 2009-000239 TOX 08508-00 Sample description: 2(5H)-Furamone, 4 (6 CAS name:

difuoroethyl)amino

CAS#: 951659-40-8

2.2. Aifluor ethyl amino furan-2 (A) IUPAC name:

Purity: Āmbie nt temperature Storage conditions Gelatine capaule Application via...: Negative control:

### 2. Test organisms

Species: Common name:

Age: Source:

Feeding during test: Standard rearing dier for quails (sshiff Spezialdiäten GmbH,

Foodinand-Gabriel-Weg 15, D-59494 Soest) Body weight at test stage 170 to 1452 gorange of both Ontrol and treatment group)

Maintenance prior to test Standard rowing der for quails (ssniff Spezialdiäten GmbH, Food:

Fordinand Gabriel-Weg 56, D-59494 Soest) Drinking water

Period of accomation to test

conditions. Starvation period prior to test sta

Mortality during acclimation period.

# B. Study design and method®

Frember 13 to October 25, 2011 1. In life da

Five birds (Featment group) were orally administered with gelatine capsules containing 2000 mg a.i./kg b.w. and were observed for a period of 28 days. In addition, 10 control birds were kept under the same circumstances from test start until day 21, where 5 control birds were sacrificed. From day 21 to 28, only 5 birds remained in the control group.

Birds were housed individually, in stainless steel wire racks which were placed indoors.

### 3. Observation and measurements

Observations on mortality and signs of intoxication were made continuously during the first 2 hours then approximately hourly on the day of dosing, afterwards at least once work-wily until test. termination.

Due to the findings on day 21, the test was prolonged to 28 days.

Body weights were recorded prior to test initiation (day -1), on study do termination).

Food consumption was measured daily until day 3, wen for the periods 3,9, 7-10,14 On study days 1, 2, 3, 7, 14 and 21 all remaining food was replaced by fresh food after cleaning. At the end of the study all surviving birds were sacriffed by  $CO_2$ out on all survivors at the end of the study.

### 4. Statistical analysis

Summarizing of raw data as well as pre-calculations (mean and standard deviation) was performed by using "Excel 2003 for Windows," of the Microsoft Corporation / USA.

RESULTS AND DISCUSSION

A. Environmental Conditions

Birds were kept under conditions which are summarised as follows:

Test temperature:

Relative humidity

21°C (mean)

Relative humidity

Relative humidit@ Photoperiod: Light intensity: Air changes:

B. Biological Findings

Mortality and behaviour:
The most noticeable effect after the application was an almost complete reduction of food consumption with all dosed birds. All further observed symptoms were consequences of this starvation, e.g. excretion of uricacid, fluffed feather of altered behaviour like reduced vigilance.

One bird started to feed in the 2 week after application, another one in the 3<sup>rd</sup> week after application. Both birds showed soft excrements of diarrhoea as indication of digestion distress. A third bird behaved similarly in the 4th week after application

Two birds Gosed God not resume feeding at all after application of the test substance. They were sacrificed for human reasons on day 28. These birds may be considered as treatment related mortalities. Since all other birds were free of symptoms or showed clear indications of recovery by that time point, the test was terminated on day 28.

Body weight development:

Due to the reduced food consumption all dosed birds showed severe body weight losses. Two birds started to regain body weight in the last week of the test.

Pathological findings at necropsy:

All birds were emaciated.

## C. Validity Criteria

The validity criterion of control mortality less than 10% w@fulfilled.

### D. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

 $LD_{50}$ : > 2000 mg a.i. Mg bod weight

### **CONCLUSION**

The acute oral LD<sub>50</sub> for BYI 02960 technical in hens (Gallus gallus gomesticus) was > 2000 mgQ.i./kg body weight.

IIA 8.1.2 Avian dietary toxicity (5-day) test in quair species or mallard duck

Report:	KIIA 8,1.2/01; T.L. (2010)
Title:	Toxicity of BY 0296 Technical During an Acute Dietary L with the Mallard
	Ducket Anas Platurbanchos) Q / V
Report No: Document No:	EBRVP020
Document No:	M-388718-01-1
Guidelines:	OECD Guideline Nov205 / V A V
\ \tag{5}	OPPTS 850.2200 V V V V
Deviations:	None A A A
GLP:	yes (corrified laboratory) A A
GLP:	yes (certified laboratory) Some data (screening of diet and water for contaminants) was not performed in
~O`	acgordance with Gor as describe tin the study report

# EXECUTIVE SUMMARY

The aim of the study was to determine the short-term effects of BYI 02960 (Sample description: TOX 0850 00 (Batch ID: 2009 000230); purity 96.2%) to Mallard Duck (*Anas platyrhynchos*).

Anas platyrhynchos (10 days old) were exposed to reated feed during a period of 5 days and observed thereafter for another 3 days while fed with unfreated feed. Nominal concentrations in feed were 313, 625, 1250, 2500 and 5000 ppm (respective mean measured concentrations: 294, 581, 1175, 2238 and 4741 ppm) which corresponded to daily uptake doses of 66, 129, 272, 459 and 825 mg a.i./kg body weight/day, respectively. To addition, unfreated diet was tested as negative control.

Mortality signs of intoxication food consumption, body weight and gross necropsy results were evaluated to determine the endpoints. No clinical signs of toxicity or mortalities were noted at any treatment level. Post-mortem examinations revealed no gross lesions or unusual observations. There was a stanstically significant reduction in Day 5 body weight and Day 0 to 5 growth at the 2238 and 4741 ppm levels, respectively, and on Day 8 for body weight at the 4741 ppm level compared to the control group. By empirical comparisons, feed consumption was less than the control group for the

4741 ppm level, and intermediate at the 2238 ppm level. Since the reduction in body weight at 2238 ppm was recovered by Day 8 the NOAEL based on body weight was 2238 ppm and the LOAEL was 4741 ppm.

The LC<sub>50</sub> was determined to be > 4741 ppm (= mg a.i./kg feed), corresponding to a  $LDD_{50} > 825 \text{ mg a.i./kg body weight/ day.}$ 

# MATERIAL AND METHODS

### A. Materials

### 1. Test material

Test item: BYI 02960 Type of test material: Substance, technical Chemical state and description: Beige powder 2009-000 9 9 Batch number: Sample description: TOX 08508-00%

CAS name: 2(5H) Furanone, 4-11(6-

difarroeth lamin@

9\$Q 659€A07-8 CAS#:

IUPAC name: #-[(6-chloropyridin

Purity:

Storage conditions:

### 2. Test organisms

Species: Common name:

Source:

Age at start of the exposure phase

Maintenance prior to test chick rearing

Temperature: Photoperio@.

Food: Trinking water: Keklad Bayer Starter Ration

Mortality:

Thre mortalities in the hate in population

Remarks: Duly birds that appeared healthy were used in the study

# B. Study design and in

# 2. Experimental treatments

Following a pre-exposure period of Gays, Dias platyrhynchos (10 days old) were offered feed treated with BYI 0290 (purity 96.2%) for 5 days In addition, untreated diet as negative control was tested. Thereafter the chief's were led with untreated feed and observed for another 3 days. Each cage (galvanized steel brooders) served as one treatment level containing ten individually marked chicks.

Per treatment evel a total amount of 11 kg feed was prepared. The test substance was rinsed into a beaker with an appropriate amount of solvent and stirred until it dissolved. The dissolved test substance was added to the corn oil and stirred. The untreated feed (i.e. raw feed) was weighed into the mixing bowl and placed on the mixer. The corn oil containing the test substance, as well as the additional

solvent rinse, was applied to the feed using a separatory funnel while the feed was mixed for a total of fifteen minutes.

### 3. Observation and measurements

Mortality and signs of intoxications were assessed approximately 1, 2 and 4 hours after diet administration on day 0, twice daily during the remainder of the study, once daily on weekends and on day 8 (study termination). Body weight measurements were conducted at day -3, day 0, day 5 and day 8. The feed consumption for each level (control and treatment birds was recorded daily during the entire period. Gross necropsies were conducted after test termination on all birds at the control and the highest test level, and on 40% of the birds in the remaining treatment levels (randomly selected). Food was analysed in order to verify the concentrations of the test item. In order to prove the stability of the test item in the brooder and in the freezer food samples were analysed for the highest and lowest treatment level (313 and 5000 ppm).

### 4. Statistical analysis

No  $LC_{50}$  (median lethal concentration) was calculated as no treatment related portalities occurred at any treatment level. For body weight and growth normality and homogeneits of variance of the data were tested using the Chi-Square Test ( $\alpha = 0.01$ ) and the Levene's sext ( $\alpha = 0.05$ ), respectively. Normally distributed data were subjected to standard one-way ANOV A followed by Dunnett's test or Bonferroni t-test.

### RESULTS AND DISCUSSION

# A. Environmental Conditions

Chicks were kept under conditions which are summarized as follows:

Room temperature during test: 22 to 37°C

Relative rumidity: 57% \$\sqrt{57\%}

Light intensity: 280 lux Ventilation of test facility 6 changes per 160 u

### B. Biological Findings

No mortality was observed during the test.

No subjethal signs of intoxications were observed

Post-mortem examinations revealed no related gross lesions or unusual observations.

Body weight at 2238 and 47 fr ppm was statistically significantly different (lower) than the control on day 5. By day 8, full recovery of the body weight reduction was observed at 2238 ppm and partial recovery was observed at 4761 ppm.

# Table: Effect of BYI 02960 on mortality, intoxication symptoms and necropsy findings of *Anas platyrhynchos*

Mean			Intoxication	n symptoms		Necropsy
measured concentration [ppm]	% mortality Day 5	% mortality Day 8	Exposure	Post- exposure	# Necropsy evaluations	Necropsý findôsgs
Control	0	0	no	no	<b>©</b> 10	none
294	0	0	no	no ,	4	none
581	0	0	no 🙈	no	4	none
1175	0	0	no 🤝	no 🐠	4 💍	none 0
2238	0	0	no "	no	4,0	none
4741	0	0	no	n&	10	🗣 nog🚱

# Table: Effect of BYI 02960 on body weight and growth of inas phytyrhynchos

Mean measured concentration	N	lean body weig [g] (mean ± \$10)		(paran	wth g]
[ppm]	Day 0	Day 5	Day 8	Exposure	Post exposure b
Control	$141.0 \pm 5.8$	261. <b>Q</b> ± 18,%	290 £ ± 26 7	<120.0 ± 16.4 €	29.2 ± 22.9
294	$136.9 \pm 9.0$	$258.3 \pm 18.6$	290.1 ±26.4	√121,5¥ 10.95	\$31.7 ± <b>2</b> 9.6
581	$139.6 \pm 7.2$	$255.8 \pm 19.7$	315.1 18.6	1162 ± 144	59:3 ¥ 23.9
1175	$136.9 \pm 6.2$	<sub>7</sub> , 254.k.≇15.8@	315.4 ± 24.2	147.2 ± 19.7	0 61.3 ± 14.3
2238	139.5 ± 11.6\$	$240.37 \pm 20.6^{\circ}$	$30\%6 \pm 24\%$	© 100.8 ± 22.4° ©	$67.3 \pm 27.8$
4741	$135.6 \pm 8.0$	$1\%6.9 \pm 1\%3^{\circ}$	<b>26</b> 0.5 ±30.3°	√ 31.3¥ 21,2©	$93.6 \pm 36.1$

<sup>&</sup>lt;sup>a</sup> The difference between Day ©and in Plation body weights.

# Table: Effect of BOI 02960 on food consumption (g per Fird per day) of Anas platyrhynchos

	N (7 Y		
Mean measured	Pre-exposure	(¹ %Exposarea	Post-exposure
concentration 🧬	[g/bird/day]	[g/bit@/day], 💯	[g/bird/day]
[pj\$@n]	(mean ± S.D.)	$(\text{mean} \pm \text{S.B})$	$(mean \pm S.D.)$
Z Č	∜day - to -1	Qay 0 to 4	day 5 to 7
Control	25.2 ± 7.5° ×	46.0 3.5	$37.6 \pm 7.9$
294	\$\frac{1}{2}\text{2.9} \pm 7.8  \text{2.9}	<b>44.4</b> ± 11.5	$40.9 \pm 11.1$
581	24.6 <b>₹8</b> .1 √	$459 \pm 12.0$	$45.0 \pm 1.4$
1175	23.0 £12.4 ×	$5.2 \pm 13.0$	$48.7 \pm 2.5$
2238	22×8 ± 7×9	$39.0 \pm 10.0$	$45.3 \pm 5.1$
47,41"	3.1 ± 26	$26.3 \pm 10.0^{b}$	$57.1 \pm 8.1$

<sup>&</sup>lt;sup>a</sup> Day 0 = first 24 hours of Goed consumption; Day 7 = second 24 hours of feed consumption; etc.

# Table: Calculation of daily dietary dose intake of BYI 02960 by Anas platyrhynchos

Mean measured A conceptration	Mean b.w. [kg b.w. brid]	kg feed/bird/day	Daily dietary dose
[ppm = mg a.i./kg Geed]	day 0 to 5	day 0 to 5	[mg a.i./kg bw/day]
\$294	0.1976	0.0444	66
5810	° 0.1977	0.0439	129
2 1 to 5	0.1955	0.0452	272
\$238	0.1899	0.0390	459
<b>©</b> 4741	0.1512	0.0263	825

<sup>&</sup>lt;sup>b</sup> The difference between termination and Day 5 body weights.

c Statistically significant difference compared to the control group

b Reduction in feed consumption as compared to the controls based on empirical analysis

Results from analytical measurements are given in the following table:

Table:	Nominal and measured diet concentrations of BVI 02960	١.

Nominal treatment level	Verification samples 🔈				
[ppm]	Measured concentration [ppm = mg a.i./kg feed]	% nominal			
Control	< 10	- 0			
313	294	94 47 5			
625	581	93 0			
1250	1175	94 9			
2500	2238	<b>90</b>			
5000	474	Ø 595 A			

# D. Validity Criteria

The control mortality was less than 10%. Measured E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived. 80% of nominal.

No LC<sub>50</sub> (median lethal concentration) was calculated, as no treatment related paortalities occurred at any treatment level. Since the reduction in body weight for the 2238 ppm treatment level was recovered by Day 8 the NOAEL based on body weight was 2238 ppm and the LOAEL was \$41 ppm.

				N C	a.
as concentration	on in f&d:	(measured cond	centration)	~Q~~	
I.C.		> 4741 <b>fypm</b> (m		, D	Ů,
$LC_{50}$ :		2 4 / 4 1 ppm (m	ıg a.l./Kg nx	ea) 🔍	<b>₹</b> ∀″ '
NOAEL		2238 ppm (mg	a.i./kgæjeeo	d) 🔎 🗆	
Α.	10,	<i>(</i> 20 4	, °~, '		W)
as daily dieta	y dose:	(based on maas	sure@conce	entration	, O
	Ĉ		L.	7 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	
LDD	, Ø ,	825 mg a.i./k	g body 😿	ight/d‱	° O .
NOĂĔL		\$25 mg a.i./k 459 mg a.i./kg	body wwig	nt/day	Z 7
		, Zi ,×			~
		W' W'	2"O"		,

CONCLUSION

The short-term effect of BYI 02960 on Mallare Duck (Anas platyrhynchos) after dietary uptake can be quantified as an LC50 of \$\infty\$ 4741 ppm (= mg/a.1./kg/leed) which corresponds to \$\infty\$ 825 mg a.i./kg body weight/day.

Report:	KIIA 8.1.2/02; , T.L. & , C.V. (2010)
Title:	Toxicity of BYI 02960 Technical During an Acute Dietary LC <sub>50</sub> with the Northern
	Bobwhite Quail (Colinus virginianus)
Report No:	EBRVP021
Document No:	M-394535-01-1
Guidelines:	OECD Guideline No. 205
	OPPTS 850.2200
<b>Deviations:</b>	None
GLP:	Yes (certified laboratory)
	Some data (screening of diet and water for contaminants) was not performed in
	accordance with GLP as described in the study report

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the short-term effects of BYI 02960 (Sample description: TOX 08508-00 (Batch ID: 2009-000239); purity 96.2%) to Northerd Boby inte Quail (Colinus virginianus).

Colinus virginianus (13 days old) were exposed to treated feed during a period of 5 days and observed thereafter for another 3 days while fed with untreated feed. Nominal concentrations in food were 313, 625, 1250, 2500 and 5000 ppm (= ms/a.i./kg/feed). Mean measured concentrations in feed were 278, 607, 1133, 2075 and 4876 ppm, which corresponded to daily watake doses of 48, 99, 170, 262 and 470 mg a.i./kg body weight/day respectively. In addition, whereated diet was tested as regative control. Mortality, signs of intoxication, food consumption body weight and gross necropsy were used to determine the endpoints.

No mortalities occurred during the study. No symptoms of toxicity were noted at any time point in any birds in the control group or at 278 607, \$\infty\$07, \$\infty\$33 and 2075 ppm, during this study. Observations of hyporeactivity, loss of righting reflex, and wing drop were noted at \$76 ppm. Post-mortem examinations revealed to gross lesions or unusual observations.

There was a statistically significant reduction in Day 5 body weight, Day 8 body weight and Day 0 to 8 growth at 2075 and 4876 ppm, respectively, compared to the control group. There was also a statistically significant reduction in Day 0 to 5 growth at 1133, 2075 and 4876 ppm compared to the control group. By empirical comparisons, feed consumption was less than the control group during the exposure period for the 2075 and 4876 ppm treatment levels, and intermediate for the 1133 ppm treatment level Since the reduction in body weight for the 1133 ppm treatment level was recovered by Day 8, the NOAEC based on bodoweight was to 33 poin and the LOAEC was 2075 ppm.

The LC<sub>50</sub> was determined to be 4876 ppm mg M./kg feed), corresponding to a LDD<sub>50</sub> 470 mg a.i.kg body weight day

MATERIAL AND METHODS

A. Materials

1. Test material



Test item: BYI 02960

Type of test material: Substance, technical Chemical state and Beige powder

description:

Batch number: 2009-000239 Sample description: TOX 08508-00

CAS name:

CAS#:

IUPAC name:

Purity:

Storage conditions:

# 2. Test organisms

Species: Common name:

Age at start of the exposure

phase: Source:

Hatching date:

Maintenance prior to test (chick rearing): Temperature:

Photoperiod: Food:

Drinking water:

Remarks:

## B. Study design and methods

1. In life dates

### 2. Experimental Peatme

days. Colinus virginianus (13 days)

days. Colinus virginianus (13 days)

edy to 25, 2009

days. To 26, 2009

days. To 26, 2009

days. To 26, 2009

greated feed and observing the study of Following a pre-exposure period of 3 days, Colinas virginianus (13 days old) were offered feed treated with BYI 02960 (purity 96.2%) for 5 dess. In addition, untreated dist was tested as negative control. Thereafter, the chicks were feed with untreated feed and observed for another 3 days. Each cage served as one treatment lever containing of individually marked chicks each.

Per treatment level a total amount of 10 kg feed was prepared. The test substance was rinsed into a beaker with 100 mL solvent and stirred until it dissolved, 110 g corn oil was weighed into a separate beaker. The dissolved test substance was added to the corn oil and stirred using a magnetic stir plate to keep it in solution. The untreated feed (i.e. raw feed) was weighed into the mixing bowl in order to obtain 11 kg treated feed per-treatment level in total. Corn oil (1%) was used as a vehicle.

# 3. Observation and measurements

Mortality and signs of intoxications were assessed approximately 1, 2 and 4 hours after diet administration of day 0, twice daily during the remainder of the study, once daily on weekends and on day 8 (study teomination). Body weight measurements were conducted at day -3, day 0, day 5 and day . The food consumption for each level (control and treatment birds) was recorded daily. All surviving firds in the control group and the high test level were necropsied. No treatment related gross lesions of unusual observations were found at the high level, therefore, only 40% of the birds in the remaining treatment levels were necropsied.

Food was analysed in order to verify the concentrations of the test item. In order to prove the stability of the test item in the brooder food samples were analysed which had been in the brooder for the highest and lowest treatment level (one day): 97 to 99%. In order to prove the stability of the test item in the freezer, food samples were analysed which had been stored in the freezer for at highest ang lowest treatment level (seven days): 92 to 100%.

## 4. Statistical analysis

No LC<sub>50</sub> (median lethal concentration) was calculated as no treatment related mortalities occurred at any treatment level. For body weight and growth, 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. Normally distributed data were subjected to standard one-way Bonferroni t-test.

### RESULTS AND DISCUSSION

### A. Environmental Conditions

Chicks were kept under conditions which are summarized as

Room temperature during test: & Brooder temperature during test: Relative humidity:

Photoperiod:

Light intensity:

Ventilation of test faci

### B. Biological Findings

No mortality was observed during the test

wing drop were noted in the 4876 ppm level. Hyporeactivity, loss

BYI 02960 on mortality, intoxication symptoms and necropsy findings of Colinus Table:

Mean measured	0 %	Intoxication	on symptoms	# Necropsy	Necropsy
concentration	Mortality Mortal	Exposure	Post-exposure	evaluations	findings
Untreated control		∡il~` ≪®ó	no	10	none
<u>√</u> 278 ≈		no	no	4	none
607		no no	no	4	none
1133		no no	no	4	none
2075		1 × 200	no	4	none
4876€	y 00 4, 0 a	yes	no	10	none
48768		V			



### Table: Effect of BYI 02960 on body weight and growth of Colinus virginianus

Mean measured		Body weight [g] (mean ±SD)		Grow (mean :	th [g] ± S.D.)
concentration [ppm]	day 0	day 5	day 8	Exposure period <sup>a</sup>	± S.D.)  Post-exposure
Control	$30.8 \pm 1.1$	$48.6 \pm 2.0$	$59.1 \pm 2.6$	$17.9 \pm 1.3$	10.4 ± 1.2
278	$32.1 \pm 1.5$	$49.4 \pm 2.6$	$60.5 \pm 3.5$	$17.2 \pm 1.7$	11.2 ± 1.6
607	$31.0 \pm 1.9$	$47.2 \pm 3.1$	$59.8 \pm 4.9$	$16.2 \pm 1.5$	12.7 2.1 11.8 ± 2.0
1133	$32.1 \pm 0.8$	$45.7 \pm 3.2c$	$57.5 \pm 2.5$	$13.6 \pm 3.5$	11,8 ± 2.0
2075	$32.1 \pm 1.3$	$36.6 \pm 3.2^{\circ}$	53.7 ± 5.0	$4.5 \pm 2.90$	10.1 ± 7.24 . 0
4876	$32.0 \pm 1.5$	$27.3 \pm 2.6^{\circ}$	$42.3 \pm 3.9^{\circ}$	-4.7 ±29°	, \$\text{Q} 5.0 \pm \text{Q} .4°

<sup>&</sup>lt;sup>a</sup> The difference between Day 5 and initiation body weights ©

### Effect of BYI 02960 on food consumption (g per bird per day) of Collinus virginianus Table:

Mean measured concentration	Feed consumption Point/do (mean ±SD)
[ppm]	day 3 to -1 day 4 to 4 Say 5 to 3
Control	49 ± 0.8 6 6 9 ± 0.9 0 6 7.4 ± 9.2
278	$\mathbb{Z}4.6 \pm 40.5$ $\mathbb{Z}$
607	$4.4 \pm 0.7$ $6.3 \pm 0.8$ $5 \pm 0.5$
1133	$7.5 \pm 1.0$
2075	4.9 ± 06
4876	$4.6 \pm 0.6$

a Day 0 = first 24 hours of reed consumption; Day 1 = second 24 hours of feed consumption; etc.

### Table:

	Daily dietary dose calculation	on
Mean measured concentration	Mead b.w. kgfood/bird/day	Daily dietary dose
[ppm]	day 0 to 5 day 0 to 5	[mg a.i./kg b.w./day]
278	$\sqrt{2} 0.0408 \sqrt{9} 0.007$	48
607	y 5 0,0391 5 5 0.0063	99
1133	0.0058	170
2075	0.0043	262
<b>48</b> 76	0.029	470

No Low (median lethal concentration) was calculated as no treatment related mortalities occurred at any treatment levels

ndings of Sylven in the following table:

<sup>&</sup>lt;sup>b</sup> The difference between termination and Day 5 body weights <sup>c</sup> Statistically significant difference compared to the control group

b Reduction in feed consumption as compared to the controls based on empirical analysis

Table: Measured diet concentrations of BYI 02960

Naminal distant	Verificat	ion samples
Nominal dietary concentration [mg a.i./kg feed = ppm]	Measured dietary concentration [mg a.i./kg feed = ppm]	% of nominal
Control	< 10	- 4
313	278	89
625	607	97,4
1250	1133	
2500	2075	<i>№</i> 3 <i>©</i>
5000	4876	\$\sqrt{98}

The control mortality was less than 10%. Measured consentrations of test item in the feed were above 80% of nominal.

E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived

No LC<sub>50</sub> (median lethal concentration) was calculated, as no treatment related mortalities occurred at any treatment level. Since the reduction in body weight for the 1133 ppm treatment level was recovered by Day 8 the NOAEL based on body weight was \$4.33 ppm and the LOAEL

as concentration in	feed:			concentrati		
LC <sub>50</sub> :		O'	> 48 <b>7</b> %/pp	m (🍿 g a.i. 🍂	g feed)	
NOAEL		~ (O)	11 <b>33</b> ppm	(mg a.i	feed)	L
Ć		Ly"			N.	Q"
as daily dietary do	<u>se:</u> 🔘 🐪	<b>)</b>	Kased Om	measured co	ncentra	(jon)
$LDD_{50}$ :		٨	> 470  mg	a i % g bodo	weigh	day
NOAEĻ 🧔	✓ ,		1704mg a.i	kg bod®w	eight/da	ıy 🦳
	.~	~ ~	U/AP *	~	~~	A 9

on Northern Bobwhite Quail (Colinus virginianus) after dietary The short-term effect ppm mg .../kg feed) which corresponds to uptake can be quantified as an DC > 470 mg a.i./10 body weight da

# Avian diefary toxicity (5-day) test in a second unrelated species

8.1.4 Subchronic and reproductive toxicity to birds Two avian dietary toxicity tests have been conducted; one with mallard duck, another with northern bobwhite quail Both studies are presented under Annex point IIA 8.1.2 above.

Report:	KIIA 8.1.4/01; , T.L., , M.T. & , C.V. (2011)
Title:	Toxicity of BYI 02960 Technical on Reproduction to the Mallard Duck (Anas
	platyrhynchos)
Report No:	EBRVP018
Document No:	M-412917-02-1
<b>Guidelines:</b>	OECD Guideline No. 206
	OPPTS 850.2300
	FIFRA Guideline 71-4
<b>Deviations:</b>	None
GLP:	Yes (certified laboratory)
	Some data (screening of diet and water for contagnants) was not performed in
	accordance with GLP as described in the study report

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the effects of dictary exposure to BY 02960 technical 02960 (Sample description: TOX 08508-00 (Batch 1D: 2009-000259); parity 96.2%) on the health and reproductive capacity of Mallard Ducks (Artas platyrhynchos).

Fifteen pairs per treatment level of Anasylatykhýnches (19 weeks old) were exposed to greated feed during a period of approximately 20 weeks. Nominal concentrations in seed were 111. \$33 and 1000 mg a.i./kg feed (= ppm) which corresponded with mean measured concentrations of 91, 298 and 845 ppm (achieved daily doses; \$28 and 81 mg a.i./kg body weight per day). In addition untreated diet was tested as negative control. &

Mortality, abnormal behaviour and signs of interaction, food consumption, body weight and gross necropsy were used to determine the endpoints for the adults. Reproductive parameters included egg number, egg shell quality, embryo wability and hatchling humber, weight, growth and survival.

No treatment related an verse effects occurred in any of the endpoints under evaluation.

The No Observed Adverse Effect Level (NOAEL) for both parental toxicity and reproduction endpoints of Mallard ducks exposed to BYI 02960 over a 20 week period was ≥845 mg a.i./kg feed (mean  $measured\ concentration), which corresponds\ to\ adaily\ dietary\ dose\ of\ \ge 81\ mg\ a.i./kg\ b.w./day.$ 

# ever (NOAEL) for both a 102960 over a 20 week penich corresponds to a daily dietan material

Test item: BYI 02960

Type of test material: Substance, technical Chemical state and description: Beige powder Batch number: 2009-000239 Sample description: TOX 08508-00

CAS name: 2(5H)-Furanone, 4-[[(6-chloro-3-pyridinyl)methyl](2,2-

CAS#:

4-[(6-chloropyridin-3-ylmethyl)(2,2-chr)uoroethyl)anoxo]furar-2(5H)-one 96.2% w/w Ambient torr IUPAC name:

None

None &

@Mallard Duck

19 Weeks

Purity: Ambient temperature Storage conditions:

2. Vehicle and /or positive control

Vehicle Positive Control

3. Test organisms

Species: Common name:

Age at start of the exposure phase:

Maintenance prior to test (chick rearing): Adult birds were housed indivers in a single room for the accommation and the study period. The adopt mallard cages measured approximately 79(L) \$61(W) x 55(M) cm were constructed of stainless steel wire grid and stainless steel sheeting. Cage floors were constructed of plastic coated steel wire and slopped to accommodate egg collection. Fach cage was equipped with a bin feed which was filled with sufficient feed on a weekly

Tekbrid Bayer Game Bird Ration

No bird mortality occurred during the acclimation period

4. Study design

Drinking water Mortality: 🗞

Food:

Replicates:

15 breeding pars per dose level; one male and one female per cage

Acclimation period:

Approx. 7 weeks proof to experimental start Temperature: @ Hatchlings: 21.6C (mean) Relative midity: Adults 54.7 % (mean)

Hatelings \$5.9 % and 57.8 % (mean)

Photoperiod: 14 h light 90 h dark

Light intensity: 8.8 to 26,2 footcandles (95 to 250 lux)

Recember 1, 2009 to June 1, 2010

Following an acclimation period of 7 weeks, Anas platyrhynchos (19 weeks old) were offered feed treated with BYI 02960; (purity 96.2%) for 20 weeks.

The dietary ingredient concentrations and quantities for the study are given in the following table:

Test level Dietary Mix								
Treatment level  [ppm = mg a.i./kg feed]	Total batch weight [kg]	Pre-mix concentration [mg/kg]	4000 mg/kg pre- mix added [kg]	Control pre-mis				
Control	20	0	0	5-4				
111	20	4000	0.56	Q				
333	20	4000	1.67	~ 0,0 ° Ø <sub>2</sub>				
1000	20	4000	5					

The exposure period was divided into a pre-photostimulation period of 8 weeks, a pre-egglaying period of 2 weeks and an egglaying period of 10 weeks. Survival and body weight of the F1-generation were observed up to two weeks after hatch. Each cage contained 1 male and 1 female. The test was conducted with 15 replicates per treatment level.

### 3. Observation and measurements

Adult body weights for the mallard reproduction study were measured at randomization on weeks 3, 5, 7 and 9, and prior to adult termination. No adult body weights were taken during the egg production phase for the study. Mortality, feed consumption and signs of intoxications were assessed daily. The food consumption was calculated from weighing the residual food produces analyzed or order to verify the homogeneity and the consentrations of the test term and its stability in the feeder.

Egg collection, candling, storage and incubation. Eggs were collected twice daily except for weekends and holidays where eggs were collected only once per day. All eggs were labeled according to parental cage and date. Eggs were retreated to revent pathogen contamination and then stored in an Egg Cooler. Any eggs that were not able to be set ("set" = transfer into the incubator) were recorded and discarded prior to egg set. Only eggs that were in good condition were used for eggshell strength and thickness measurements.

Egg incubation was initiated weekly (after start of reproduction). Or Day 23 of incubation, the eggs were allowed to hatch:

Hatchling body weights were measured and recorded on the day they were removed from the hatcher and on Day 14. Hatchlings were observed once dails throughout the 14-day period for signs of toxicity, injuries, illness and mortality. Mortalities that occurred prior to the end of the 14-day period were recorded and discarded. Hatchlings that survived the 14-day observation period were sacrificed by CO<sub>2</sub> asphyxiation, weighed, and discarded.

### 4. Endpoints

Adult Body Worth: The adult body weight change from initiation of dosing to adult termination, analyzed on or per cage basis by sex.

Adult Feed Consumption: The means were calculated as grams/bird/day, analyzed on a per cage basis.

Eggs Laid per hen: Analyzed as the total egg production, on a per hen basis.

Eggs Cracked eggs as determined by candling prior to incubation, analyzed on a per hen basis.

Eggs Not Cracked of Laid (%): Eggs laid that were not cracked as a percentage of eggs laid on a per hen basis.

<u>Eggshell Strength:</u> The force needed to penetrate the shell and membrane measured at one point on the waist of the egg.

Eggshell Thickness: The thickness of the shell plus the membrane measured at three points around the waist of the egg.

Eggs Set: Eggs placed under incubation are "set", analyzed as total number on aper hen basis

<u>Viable Embryos (mean):</u> Fertility is determined by candling on the 11<sup>th</sup> or 12<sup>th</sup> day of incubation. Fertility analyzed as total fertile eggs.

<u>Live Embryos (mean)</u>: Live embryos are determined by candling on the 8<sup>th</sup> or 19<sup>th</sup> day of incubation Eggs Set of Laid (%): Eggs set as a percentage of eggs aid on a per ten basis.

Viable Embryos of Eggs Set (%): Live embryos as precentage of eggs set on aper her basis

Live Embryos of Viable Embryos (%): Live embryos as a percentage of viable embryos on a per hen basis.

Number Hatched (mean): Live hatchlings that had berated themselves from their eggs by des 28 of incubation. Analyzed as the total number of normal hatchlings on a per hen basis.

Number Hatched of Live Embryos (%) Hatchlings as a percentage of live embryos on open ben basis.

14-day-old Survivors (mean): Live Phicks at 14-days post hatch

14-day-old Survivors of Eggs Sel (%): Tive chicks at 4-day post Patch as a percontage of eggs set on a per hen basis.

Number Hatched of Eggs Land (%). Hatchings a percentage of eggs laid on a per hen basis.

Number Hatched of Eggs Set (5): Hatchlings as a percentage of eggs set on a perhen basis.

14-day-old Survivors of Number Harched (%): Live chiefs at 14-days post hatch as a percentage of hatchlings on a percentage basis.

Hatchling Bodo Weight Indiodual Weights of the five hatchlings taken upon removal from the hatcher, analyzed on a per her basis.

14-day-old Survivor Body Weight: In Evidual weights of the 14-day-old offspring taken at sacrifice, analyzed on a per hen basis.

### 5. Statistical analysis

For avian reproduction data normality and homogeneity of variance of the data were tested using the Shapiro-Wilk's Test ( $\alpha \neq 0.01$ ) and the evenes test ( $\alpha = 0.05$ ), respectively. Normally distributed data were subjected to standard one-way anolya followed by Dunnett's test or William's-test. Non-parametric analyses were conducted using the Lonckheere or Mann-Whitney procedures.

Analysis of adultærmination bodyweight data was conducted by subjecting data to a Chi-Square Test for normality and a Bartlett's rest for equal variances. The analysis of variance was conducted followed by a Dunnett's Test for unqual replicate size.

# RESULTS AND DESCUSSION

A. Environmental Conditions

Test animals were kept under conditions which are summarised as follows:



Room temperature during test:	21.3 °C			
Relative humidity:	54.7%			_@ °
Photoperiod:				
Acclimation and pre-photostimulation period:	7 hours ligh	t/17 hours dark	A	
Reproduction period:	17 hours lig	ht/7 hours dark		
Light source	Fluorescent	lamp	T'	~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Light intensity:	95 to 250 lu	X	.1	S S
Ventilation of test facility:	15 air chang	ges per hour	<b>J</b>	
Brooder:			¥	
Temperature:	36.9 °C <sup>™</sup>	, Q		
Air humidity:	59.8%	ŽO,	W	
Rotation of eggs:	Approximat	ely everotwo h	ours 🎺 🔏	* & .@
Hatching compartment:	~ *		Q',ô	
Temperature:	. 36.8 °C 。			
Air humidity:	<b>%</b> 69.3%			*\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\
Pen (for hatchlings):		Ö Ö T	\$0, Q, Y	
Photoperiod:	14 bours lig	ht / 10 Hours da	rk 🔊 🔘	
			, O' &	
Diplominal Findings				
5. Diological Findings		, W		,
No adult mortality or other effects of parenta	l health occur	ed at any treat	men# level Sev	erad adult
airds were observed in the control and treatm	ent@evels@ith	feather loss a	nd Okin abrasio	ns/lacerations
	· · · ·		iio skiii dordsio	113/140014110113
	r bareda Nilla atate	Sation 11 a Ser his	lagically giand	Vaant affaata
is a result of normal cage wear for laboratory	birds. No stat	stically or big	logically signif	cant effects
is a result of normal cage wear for laboratory vere observed in the reproductive endpoints?	birds. No state	Asticallo or big	logically signif	icant effects
vere observed in the reproductive endpoints?	birds. No state	sticalls or big	Jogically sign	Tcant effects
yere observed in the reproductive endpoints.	birds. No state	Extically or big	Jogically signed	fcant effects
ere observed in the reproductive endpoints?  Summary of reproductive perf	ormanic of An	stically or big	logically signed	YI 02960
vere observed in the reproductive endpoints?  Summan of reproductive perf	ormance of And	stically or big  strong by the	logically signal  s treated with B  2333 ppm	YI 02960
vere observed in the reproductive endpoints.  Summary of reproductive performance of replicates.	control  15	stically or big strong by the	ogically signal  s treated with B  333 ppm  15	YI 02960  1000 ppm  15
vere observed in the reproductive endpoints.  Summary of reproductive performance of replicates.  Total eggs laid	ormance of 4/10 Control 15	stically or big strongly of the strongly of th	s treated with B  333 ppm  15  53.7	YI 02960  1000 ppm 15 61.3
rable:  Summary of reproductive perf  Number of replicates  Total eggs laid  Eggs cracked	contract  Contra	stically or big s plat@hynches 12 12 55 00.27	s treated with B  333 ppm  15  53.7  0.33	YI 02960  1000 ppm 15 61.3 0.21
rable:  Summary of reproductive performance of reproductive performance of reproductive performance of reproductive performance of replicates.  Total eggs laid  Eggs cracked  Eggs set	Control  15  616  755.9	**Stically or big **Strically or big **Strically or big **Strically or big **Strict	s treated with B  333 ppm  53.7  0.33  47.5	YI 02960  1000 ppm 15 61.3 0.21 55.4
vere observed in the reproductive endpoints.  Summan of reproductive perf  Number of replicates  Total eggs laid  Eggs cracked  Eggs set  Viable Embryos	Control  15  20  20  55.9	**Stically or big **Is plate hyncho  **Extra	s treated with B  2333 ppm  15  53.7  0.33  47.5  42.8	YI 02960  1000 ppm 15 61.3 0.21 55.4 51.4
vere observed in the reproductive endpoints.  Summan of reproductive perf  Number of replicates  Total eggs laid  Eggs cracked  Eggs set  Viable Embryos  Live 3-Week Embryos	Control  13  60  60  70  70  15  70  70  70  70  70  70  70  70  70  7	(s plato hyncke (s plato hyncke (1 ppm 1 0 50.5 0.27 0 50.37 48 8	s treated with B  2333 ppm  15  53.7  0.33  47.5  42.8  42.5	YI 02960  1000 ppm 15 61.3 0.21 55.4 51.4 51.1
Summan of reproductive performance of reproductive performance of replicates  Total eggs laid  Eggs cracked  Eggs set  Viable Embryos  Live 3-Week Embryos  Hatchlings	Control  15  0.20  55.9  51.5  47.5	**Stically or big **Is platohyncko **End of the state of	s treated with B  333 ppm  15  53.7  0.33  47.5  42.8  42.5  37.7	YI 02960  1000 ppm 15 61.3 0.21 55.4 51.4 51.1 46.3
Hatchlings	Control  15  0.20  55.9  51.5  47.5  99.7	**Stically or big **Is plate hynchro **End of the plate hynchro **End of th	s treated with B  333 ppm  15  53.7  0.33  47.5  42.8  42.5  37.7  99.5	YI 02960  1000 ppm 15 61.3 0.21 55.4 51.4 51.1 46.3 99.7
Eggs Not Crocked/Engs I add (%)	√ 99.7 <sup>U</sup>	<b>99.3</b>	99.5	99.7
Eggs Not Cracked/Eggs Lad (%) Viable Embryos/Eggs Se (%) Live 3-Week Embryos/Viable Embryos/(%)	99.7 99.7 99.7	99.3 96.7	99.5 87.3	99.7 93
Eggs Not Cracked/Eggs Lad (%) Viable Embryos/Eggs Se (%) Live 3-Week Embryos/Viable Embryos/(%)	99.7 99.7 99.7	99.3 96.7 98.0	99.5 87.3 99.2	99.7 93 99.3
Eggs Not Cracked/Eggs Lad (%) Viable Embryos/Eggs Se (%) Live 3-Week Embryos/Viable Embryos/(%)	99.7	99.3 96.7 98.0 90.3	99.5 87.3 99.2 88.8	99.7 93 99.3 89.4
Eggs Not Cracked/Legs Lad (%) Viable Embryos / Eggs Set (%) Live 3-Week Embryos / iable Embryos (%) Hatchlings/Live 3-Week Embryos (%)	99.7 99.7 99.5 99.5 99.5	99.3 96.7 98.0	99.5 87.3 99.2	99.7 93 99.3

Table: Egg shelkthickpess of Anas playrhynchos treated with BYI 02960

Nominal treatment level	Shell thickness (mean ± SD)	Shell strength (mean ± SD)
[ppm] ~ ~	[mm]	[kg]
~ control 4	$0.363 \pm 0.018$	$2.66 \pm 0.28$
11107	$0.355 \pm 0.020$	$2.72 \pm 0.39$
25 30 0 ° 4	$0.359 \pm 0.018$	$2.55 \pm 0.30$
<b>\$</b> \$\text{\$000}	$0.369 \pm 0.014$	$2.82 \pm 0.27$

Table: Body weight of hatchlings of *Anas platyrhynchos* treated with BYI 02960

Nominal	Hatchlings		14 day old survivors		0 Q1
treatment level [ppm]	Number	Body weight (mean ± SD) [g]	Number	Body weight (mean ± SD) [g]	% Mortality
control	712	$36.3 \pm 2.1$	708	$269.8 \pm 17.5$	4 , 5
111	649	$35.3 \pm 1.9$	646	$268.9 \pm 13.8$	
300	566	$36.3 \pm 2.8$	564	273.4 ± 4.0	2 🛒 2
1000	648	$35.0 \pm 1.7$	646 <b>©</b>	266.4 15.4	

# C. Analytical Findings

Analysis of test diet: The nominal amounts of BVI 02960 technical in the feed were 0 (control), 141, 333 and 1000 ppm (= mg a.i./kg feed), respectively. The average measured amounts of BVI 02960 technical for week 1, 5, 10, 15 and 20 were determined as 0 (control), 21, 298 and 845 ppm, respectively, representing percent of nominal values of 82, 90 and 85%, respectively.

Homogeneity of mixing was verified with the 111 and 1000 ppm treatment levels. Tripscate samples were obtained from the top, middle and bottom of the mixing versel. The average measured amounts of BYI 02960 technical for the nominal amounts of 111 and 1000 ppm were determined as 108 ppm (RSD = 13%) and 1001 ppm (RSD = 8%), respectively.

Freezer stability samples were stored in a freezer for approximately five weeks and analyzed for BYI 02960 for the nominal treatments of 111 and 1000 ppm. The measured amounts of BYI 02960 for the treatments were 86 and 930 ppm, respectively. The 5 week values were compared to the initial Week 1 measured analysis and percent initial values were determined as 81 and 93% for the 111 and 1000 ppm treatments, respectively.

Room stability camples remained for seven days under study room conditions and were analyzed for BYI 02960 for the nominal treatments of 111 and 1000 ppm. The measured amounts of BYI 02960 for the treatments were 82 and 930 ppm, respectively. The 7 day stability sample values were compared to the initial Week 1 measured analysis and porcent initial values were determined as 78 and 93% for the 111 and 1000 ppm treatments, respectively

Results from analytical measurements are summerised as follows:

Table: Measured dict concentrations of BX 02960

Ê

& · ·	~ (	<b>*</b>					
Nominal	Week 1 ®	Week 5	Week 🐠	Week 15	Week 20	Mean	Percent of
dietary	<i>\(\)</i>					Measured	Nominal
concentration	/ <u>_</u>		**			Values (±SD)	
Control	& JOQ	< L/QQ .	LOQ	< LOQ	< LOQ	< LOQ	n.d.
111 ppm 🧳	\$106 C	<b>3</b> 3	° 63	89	106	91.4 (17.7)	82
333 ppm	270	278	269	332	343	298.4 (36.1)	90
1000 ppm	999	<b>∜</b> 730	779	999	718	845.0 (142.4)	85

## Table: Calculation of the daily dietary dose of BYI 02960 based on 22-week food consumption

Measured dietary	Body weight at randomization	Body weight at termination	Mean body weight	Food consumption	Daily
concentration	[kg]	[kg]	[kg]	[kg feed/bird/day]	dietary dose [mg a.i. kg
91 ppm	1.04	1.156	1.098	0.105	b.w.@yy]
298 ppm	1.043	1.168	1.106	0.103	28
845 ppm	1.017	1.11	1.064	102	818

# Table: Homogeneity and analytical verification of concentrations of BYI 02960

Nominal	Homogene	Homogeneity in diet Verification of concentrations in die (% recovery)			Verification of concentrations in				
dietary concentration	mean	% ev	Week 10	Week 5	Week 10	Week 35	Week 20		
111 ppm	108	13%	<b>9</b> .5	° 83	€ 60 € 0	<b>30</b> %	9.6		
333 ppm	_*	_*	<b>8</b> 1 <b>¢</b>	841	\$\ 81\@	₩00 g	<sub>4</sub> 103		
1000 ppm	1001	8%	100	Ø3 &	) 7 <b>8</b> °	1000	\$\tag{72}\$\$\\\$\\\$\\\$\\\$\\\$\\\$\\\$\\\$\\\$\\\$\\\$\\\$\		

<sup>\*</sup> Homogeneity was tested only for nominal concentrations of 11.1 and 4000 ppm, respectively

# Table: 7-day ambient and freezer stability of BYI 02960

Nominal	7-day roo	m <b>©</b> ability©	2 6	Freezer s	
dietary	Mean measured 🦠	Mean percen	nt of M	lean measured	Mean percent of
concentration	[ppm a(i)]	nominal		[ppm a2.]	O day 0
111 ppm	8 <b>2</b> ,3	, TO	10	<b>86</b> 23	, © 81
1000 ppm	~930 q	Q 333		932	93

### D. Validity Criteria

The control mortably was less than 10%. Measured concentrations of test item in the feed were above 80% of nominal The shell thickness of eggs from the controls was above the species-specific threshold. The average number of 14-day-old survivors per ben in the controls was above the species-specific threshold.

# E. Biological Endpoints Derived

From the results presented above the following biological ordpoints can be derived:

as concentration in feed:  $\sim$  homing concentrations NOAEL  $\geq$  1000 ppm ing a i0kg feed LOAEL > 1000 ppm (mg a)/kg feed)

as concentration of feed: (based on measured concentrations)

NOAEL LOAEL  $\geq$  845 ppm (mg a.i./kg feed)  $\geq$  845 ppm (mg a.i./kg feed)

as daily thetary these: (based on measured concentrations)

NOART > > 81 mg a.i./kg bw/day> 81 mg a.i./kg bw/day

### CONCLUSION

The effect of BYI 02960 on the reproduction of Mallard Duck (Anas platyrhynchos) after dietary uptake can be quantified as a NOAEL of  $\geq$  845 mg a.i./kg feed which corresponds to  $\geq$  81 mg a.i./kg b.w./day.

b.w./day.	
Report:	KIIA 8.1.4/02; , T.L., , M,T. & , C.X (2012)
Title:	Toxicity of BYI 02960 Technical on Reproduction to the Northern Brownite Quail (Colinus virginanus)
Report No:	EBRVP019
Document No:	M-424704-01-1
Guidelines:	OECD Guideline No. 206 OPPTS 850.2300 FIFRA Guideline 71.4
	OECD Guideline No. 206 A OPPTS 850.2300 FIEDA Cuideline 71.4
<b>Deviations:</b>	None of G & A A A A
GLP:	Yes (certified laboratory)

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the effects of dietary exposure to BYI 02960 technical@Sample description: TOX 08508-00 (Batch D: 2009-000239); purity 96.2%, on the health and reproductive capacity of Northern Bobwhite Quail (Colinus Virginagius).

Eighteen pairs of birds per treatment of Colorus virginiams (18 weeks old) were exposed to treated feed during a period of 23 weeks. Nominal concentrations in feed were 111 \$\infty\$33 and 1000 mg a.i./kg feed (=ppm) which corresponded to mean measured concentrations of 107, 302 and 999 ppm and achieved daily doses of 14, 40 and 154 mg &i./kg body weight per day, Despectively.

Birds were observed for nortality abnormal behavior and signs of toxicity adult body weight and feed consumption were measured; gross pathology was conducted; reproductive parameters, as well as hatchling health, growth and surviyal, were examined.

At 1000 ppins, there were statistically and biologically significant offects on parental survival, health and body weight, and subsequently on several reproductive parameters. At 300 ppm, there were statistically significant but small effects on several parameters that were not considered biologically relevant.

Therefore, the 6 Observed Adverse Effect Level (NOAEL) for both parental toxicity and reproduction endpoints of bobwhite quadexpoord to BVI 02960 over a 23-week period was 302 mg a.i./kg feed (ppm), which corresponded to fing a 4./kg h.w./day, MATERIAL AND METHODS

A. Materials

1. Test material

Test item: BYI 02960

Type of test material: Substance, technical Chemical state and description: Beige powder Batch number: 2009-000239 Sample description: TOX 08508-00

CAS name: 2(5H)-Furanone, 4-[[(6-chloro-3-pyridinyl)methyl](2,2-

difuoroethyl)amino]

CAS#: 951659-40-8

4-[(6-chloropyridin-3-ylmethyl)(2,2-diffuoroethyl)amoro [fura IUPAC name:

2(5H)-one

96.2% w/w Purity:

Approved until Storage conditions:

Vehicle and/or positive control: None

2. Test organisms

Species: Colina virginanus Northern Bobwhite Juail Common name: 18 weeks∾

Age at start of the exposure phase:

Source

Food:

Maintenance prior to test (chick rearing):

Housing:

Adult birds were housed indoors in a single room for the @ acclimation and the study period. The adult qual cages measured approximately 6(L) x 28(W) 27(H) m were constructed of stainless stee wire stud and rainless steel sheeting. During the feeding period, additional feed was weighted and added to the bin ferders anneeded!

Teklad Bayer Same Rord Ration

LocaDtap water

No bird mortality occurred during the acclimation period

Mortality: 3. Study design

Drinking water:

18 breeding pairs per dos level; one male and one female per cage Replicates:

Approx 4 week prior to experimental start

Acclimation period: Adults: 21.3% (mean) Temperature:

Hatchlings 22.2°C (mean) Relative humidity Agults: 55.3 % (mean) 🔬 Matchlings: 570 % (mean)

Adults: 7 h light: 17% dark (acclimation and short day length Photoperiod:

phase (8 weeks))

17 h light: 7 h dark (remainder of the study)

Matchtongs: 14th light: 10 h dark

Adults: 64 to 194 lux Hatchlings©252 lux

B. Study design and

ovember 01, 2010

Following an acclimation period of 4 weeks, Colinus virginianus (18 weeks old) were offered feed treated with BYI 02960; (purity 96.2%) for 23 weeks.

The dietary ingredients and quantities for the study are given in the following table:

**Test Level Dietary Mixing** 

Nominal Treatment Concentration [ppm]	Total Batch Weight [kg]	Pre-Mix Concentratio n [mg/kg]	2000 mg/kg Pre-Mix added [kg]	Control Pre- Mix added [kg]	Amount of Raw Feed [kg]
Control	11	0	0	5.5	5.5
111	11	2000	0.61	0 💸	10.30
333	11	2000	1.83	0 🔊	9.17
1000	11	2000	5.5	<sub>4</sub> 0 °	\$5.5 ×

Eggs were collected twice daily from the parental birds daying the exposure phase. The exposure period was divided into a short day length period of 8 weeks and a long day length period of 15 weeks. Effects on adult survival, health, body weight, and feed consumption were evaluated. In addition, the effects of adult exposure to BYI 02960 technical on the number of eggs late, fertility, embryo wiability, hatchability, offspring survival, and eggshell quality (strength and thickness) were evaluated. Each cage served as one replicate containing 1 male and Pfemale. The test was conducted with 18 teplicates per dose.

## 3. Observation and measurements

Adult body weights and feed consumption for the quail reproduction study were measured at randomization, on weeks 3, 5, 7 and 9 and prior to adult termination. No adult bedy weights were taken during the egg production phase for the study. Adult feed consumption was measured weekly by cage throughout the study and calculated from weighing the residual food. Food was analysed in order to verify the homogeneity and the concentrations of the lest item and its stability in the feeder.

Egg collection, canding, eggshell evaluation, storage and incupation. Eggs were collected twice daily except for weekeness and holidays where eggs were collected only once per day. All eggs were labeled according to parental cage and date. Eggs were treated to prevent pathogen contamination and then stored in an Egg Cooler. Any eggs that were no able to be set ("set" transfer into the incubator) were recorded and discarded prior to egg set.

Eggshell strength and thickness was measured each week during the egg laying phase. One egg was collected from each of the odd numbered cages during odd numbered weeks (i.e. 1, 3, 5, 7, and 9) and one egg was collected from each of the even numbered cages during even numbered weeks (i.e. 2, 4, 6, 8, and 10). Only eggs that were in good condition were used for eggshell strength and thickness measurements.

Egg incubation was initiated weekly (after start of Opproduction). On Day 21 of incubation, the eggs were allowed to hatch.

Hatchling bodoweights were measured and recorded on the day the hatchlings were removed from the hatcher and on Day 14. Feed consumption for the offspring was not monitored. Hatchlings were observed once daily throughout the 14-day period for signs of toxicity, injuries, illness, and mortality. A record was maintained of all observations. Mortalities that occurred prior to the end of the 14-day period were recorded and discarded. Hatchlings that survived the 14-day observation period were sacrificed by CO<sub>2</sub> asphyxiation, weighed, and discarded.

Food was analysed in order to verify the homogeneity and the concentrations of the test item and its stability in the feeder.

#### 4. Endpoints

Adult Body Weight: The adult body weight change from initiation of dosing to adult termination Analyzed on a per cage basis by sex.

Adult Feed Consumption: The means were calculated as grams/bird/day, analyzed on a per calculated as Eggs Laid per Hen: Analyzed as the total egg production, on a per hen basis \( \)

Eggs Cracked of Eggs Laid: Cracked eggs are determined by candling proof to incubation a per hen basis.

Eggs Not Cracked of Laid (%): Eggs laid that were no cracked as percentage of Eggs laid on a hen basis.

Eggshell Strength: The force needed to penetrate the shell and membrane measured at one point on the waist of the egg.

Eggshell Thickness: The thickness of the shell plus the membrane measured at three points around the waist of the egg.

Eggs Set: Eggs placed under incubation are "set", analyzed as total number on a per hear basis

Viable Embryos (mean): Fertility is determined by candling on the Fertility analyzed as total fertile eggs.

Live Embryos (mean): Live embryos are determined by candling on the 18th or 19th day of incubation.

Eggs Set of Laid (%): Eggs set as a percentage of eggs laid on a per Ken basis.

Viable Embryos of Eggs Set (%). Live embryos as apercentage of eggs set on a per hen basis.

<u>Live Embryos of Viable Embryos (%):</u> Live embryos as a percentage of viable embryos on a per hen basis.

Number Hatcher (mean): Live hatchings, which had liberated themselves from their eggs by day 28 of incubation. Analyzed as the total number of normal hatchlings on a per hen basis

Number Harched of Live Embros (% Hatchlings as a percentage of live embryos on a per hen basis.

14-day-old Survivors (mean); Live chicks at 14-days post hatch

Live chicks at 14-dax post hatch as a percentage of eggs set on a per hen basis.

(%) HatoQings as a percentage of eggs laid on a per hen basis.

Number Harched of Egg@Set (%): Hatchlings as a percentage of eggs set on a per hen basis.

14-day-old Survivors of Number Hatched (%): Live chicks at 14-days post hatch as a percentage of hatchlings on a per hen basis.

Hatchling Body Weight. Individual Weights of the live hatchlings taken upon removal from the hatcher, analyzed on wer hen basis

14-day-old Survivor Body Weight: Individual weights of the 14-day-old offspring taken at sacrifice, analyzed on a per hen basis.

#### 5. Statistical analysis

The no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) was identified for each parameter using hypothesis testing methodology. All hypotheses testing were

performed with a specialized statistical program designed to analyze avian reproduction data (EFED). All data was analyzed independently according to each end-point. Data from treatment groups were compared to controls using the Shapiro-Wilk's test for normality and Levene's test of equal variance to determine if dose groups had unequal variances. If assumption of normality ( $p \le 0.01$ ) and homogeneity of variance  $(p \le 0.05)$  were met, then parametric analyses were conflicted using abalysis. of variance (ANOVA) followed by Dunnett's test or William's test. If variances were unequal, then the non-parametric analyses were conducted using the Jonckheere or Mann-Whitney procedures. Statistical analyses were performed using SAS® statistical configuration of the statistical computers with conclusions of statistical significance at the  $\alpha = 0.05$  (95% confidence evel).

#### RESULTS AND DISCUSSION

#### A. Environmental Conditions

Test animals were kept under conditions which are

Room temperature during test: tion period: Relative humidity: Acclimation and pre-photostimulation Reproduction period 7 hours light/17 hours dark Juurs Oght/7 h 17 hours Oght/7 hours dank Light source Light intensity: Ventilation of test facility Brooder: Temperature: Air humidity: Č Rotation of sess: Hatching compartment Temperature: Air humidity: Pen (for hatchlings) hours light / hours dark Photoperiod: B. Biological Findings

Adult mortality

Nine adult mortalities occurred during the test with two birds in the control group, one bird in the

name additional times occurred of times with two birds in the control group, one bird in the 111 ppm level, and six birds at 1000 ppm. No adult mortalities occurred in the 333 ppm treatment level.

Table:	Adult mortality of <i>Colinus virginianus</i> treated with BYI 02960

	Adult Bird Mortality Summary					
Cage	Bird	Bird	Mortality	Final Bird	Observation	
No.	No.	Sex	Date	Weight [g]	Observation	
Control						
002	218	Female	05-Sep-10	251	Feather loss on head	
008	831	Male	26-May-10	193	None ~	
				111 ppm		
103	734	Male	14-Sep-10	150	Feather loss on head, abrasion on feet	
				1000 ppm 🧸		
303	814	Male	04-Jul-10	139	Lacerations on feet, emaciated	
307	302	Female	15-Sep-10	124	Laceration on foot, feathor loss on breaso	
308	273	Female	10-Sep-10	129	Feather loss of back, laceration on foot and bower hack, emacrated	
310	330	Female	10-Sep-10	0141	Feather Moss on back wing, and neck lace and leg maciated	
313	277	Female	06-Sep-10		Feather loss on pock, back, and log, laceration on toot, toos, and head	
315	271	Female	07-Sep	¥ 107\$	Feather loss on breast, lacerations on feet,	

The mortalities at 1000 ppm were considered treatment related following necrops observations. Four female birds in the 1000 ppm treatment level were observed as being emaciated with all five female birds either having regressed ovaries, no maturing follicles, or reduced egg laying.

Thus, the LOAEL for parental survivation beconsidered at the top dose level (1000 ppm). The related NOAEL is 333 ppm.

### Adult bird feed @nsumption

There were no statistically or biologically significant differences in the feed consumption to the control at any treatment level. Thus, the LOAEL for adult feed consumption can be considered at above the top dose level (> 1000 ppm). The clated NOAEL is 1000 ppm.

Table: Adult feed consumption of Coltius virginianus treated with BYI 02960

Nominal Treatment	Adult feed consump	ton (g/bird/day)
[apm]	(mean ± S.D2)	% reduction
Control	Q 27.2 ± 3.6	-
111	26.2 # 8.1	3.40
333	\$\infty \ \tag{28.6} 5.1 \\$\infty \	-4.16
1000	25,7 ± 3,4 °	5.43

Adult body weight and body weight gain

There was a statistically significant effect for the adult male body weight gain at the 333 ppm test level, however the magnitude of the difference to the control bodyweight remained < 10%, and no dose-response was apparent, so that the biological significance was considered to be low. There was a statistically significant effect on the adult female body weight gain at 1000 ppm that was related to a biologically significant difference in body weight. No effects were observed on adult male and female body weight in any other test levels. Thus, the LOAEL for parental body weight can be considered at the top dose level (1000 ppm). The related NOAEL is 333 ppm.



Table:	Adult male body weight and bodyweight gain of Colinus virginianus treated with BYI 02960

Nominal Treatment	Bodyweig (mean ±		Bodyweight gain [g] (mean ± S.D.)
[ppm]	Week 1	Termination	(mean ± S.D.)
Control	$202 \pm 11.0$	$222 \pm 20.3$	$12.7$ $\checkmark$ $\checkmark$
111	$197 \pm 9.4$	$207 \pm 16.4$	10.4 ± 14.5
333	$200 \pm 9.6$	$209 \pm 20.7$	$4 \times 15.9 \pm 20.0 $
1000	$195 \pm 11.3$	203 ± 18.59	9.6 ± 10.8 V
* Statistically signific	ant <sup>B</sup> Biologically significa	ant C	

<sup>\*</sup> Statistically significant

#### Adult female body weight and bodyweight gain of Colinus virginianus reated with BYI Table:

Nominal Treatment		Godyweight [g] (mean ± S.D)	Bodyweigh	gain [g]
[ppm]	Week 1	Termination		
Control	$199 \pm 7.2$	234 ± 350	$35.0 \pm 3$	34.0
111	$199 \pm 7.5$	233 + 27.3	37.0 ±3	!5.9 ≼, 🔊 '
333	$199 \pm 7.2$	2394 18.5	42.10 1	3.6 \$
1000	$199 \pm 7.0$	204 297	Ø _\$ 10,\$± 26	5, <b>2</b>

<sup>\*</sup> Statistically significant

#### Adult bird necropsy

Necropsy of all adult birds to study termination showed an apparent treatment effect at the 1000 ppm level. Adult female birds in the 1000 ppm treatment level were observed with the following:

8 birds with regressed waries which were higher as opposed to other treatment levels; 10 birds with a lower number of matering follicles than in the other treatment levels, and 5 eggs found in the oviduct which were lower than the other treatment levels.

In addition, a higher number of birds in the 1000 from treatment Devel skin lesions/abrasions and emaciated birds as compared to the other treatment levels. Feather loss was noted for birds in all treatment levels due to normal sage wear for laboratory reared qual.

No other effects attributable to treatment with BY 02960 were observed at any other treatment level. Thus, the LOAEL for parental health based or necropsy results can be considered at the top dose level (1000 ppm). The related NOAFI

#### Egg endpoints

Egg number: there were statisticalloand biologically significant differences at the 1000 ppm treatment level as compared to the control for the number of eggs laid and the number of eggs set. There was a statistically significant difference at the 333 and 1000 ppm treatment levels as compared to the control for the percent eggs, set of eggs laid, however these differences were not considered biologically significant as the reduction was 1.89% and 3.62%, respectively.

The LOAEL for the egg endpoints was determined at the top dose level (1000 ppm). The related

<sup>&</sup>lt;sup>B</sup> Biologically significant

Table:	Egg number of <i>Colinus virginianus</i> treated with BYI 02960
--------	---

Nominal	Nominal No. of eggs laid		No. of eggs laid No. of eggs set		% Eggs set of eggs laid	
Treatment	mean ± SD	%	mean ± SD	%	mean ± SD	% &
[ppm]		reduction		reduction		reduction
Control	$55.6 \pm 16.5$	-	$50.1 \pm 16.6$	-	88.7	
111	$51.1 \pm 13.0$	8.15	$44.3 \pm 12.5$	11.62	86.3€ 5.3	<b>2</b> 78
333	$60.8 \pm 9.2$	-9.42	$53.1 \pm 8.9$	- 5.87	$87.9 \pm 3.5$	1.89*
1000	$30.9 \pm 16.4 *^{B}$	44.4	$26.6 \pm 14.7 *^{B}$	46.85	\$5.5 ± 3.1	3.62

<sup>\*</sup> Statistically significant

#### Eggshell quality

There were no statistically significant differences at any treatment level as compared to the control for eggshell thickness or eggshell strength. The LOAD for the eggshell quality end-points was considered at above the top dose level (> 1000 ppm). The related NO AEL  $\approx 2000$  ppm.

Egg shell quality of Colinus pirginianus treated with BYI 02960. Table:

Nominal Treatment [ppm]	Shell thickness (mean ± SD) Shell strength (mean ± SD) [kg]
control	$0.21 \pm 0.01$
111	$9.22 \pm 0.17$
300	$0.21 \pm 0.01$ % $0.09 \pm 0.09$
1000	$0.22 \pm 0.01$ $0.79 \pm 0.13$

#### Embryo endpoints

There were statistically and biologically significant differences at the 1000 ppm treatment level as compared to the control for the number of viable embryos and the number of live embryos.

There were no statistically significant differences at any treatment level as compared to the control for percent viable embryos of eggs set and live embryos of viable embryos. The LOAEL for the embryo endpoints was determined at the top dose level (1000 ppm) but the effect is primarily due to a lower number at eggs laid or set, rather than to reduced enobryo surviva PThe related NOAEL is 333 ppm.

Embryo endpoints of Colinus virginianus treated with BYI 02960 (absolute) Table:

Nominal Treatment	nt No. of live embryos No. of live embryos		e embryos	
[ppm]	mean⊕SD &	% reduction	mean ± SD	% reduction
Control	\$\tilde{\chi}\$ 44.\$\tilde{\chi}\$ 17.9\$\tilde{\chi}\$		$44.3 \pm 17.7$	
111		<i>®</i> ≈ <b>⊅</b> 070	$42.5 \pm 12.6$	3.98
333 📣 🐇	3 ± 100.0	10.12	$48.9 \pm 10.1$	-10.36
1000	4.8 ± 4.9 *B	<sup>2</sup> 44.56	$24.7 \pm 14.7^{*B}$	44.17
* Statistically sign@can	t B Biologically signific	Ç <b>Q</b> ÎT		

<sup>\*</sup> Statistically sign@c

<sup>&</sup>lt;sup>B</sup> Biologically significant



Table:	Embryo endpoints of <i>Colinus virginianus</i> treated with BYI 02960 (relative)	

Nominal Treatment [ppm]	Viable embryos of eggs set		Live embryos of viable embryos	
[bbiii]	mean ± SD	% reduction	mean ±SD	% reduction
Control	$91.4 \pm 21.1$	-	99.0	, - Ö
111	$96.6 \pm 5.0$	-5.65	99.0	0.02
333	$94.2 \pm 15.8$	-3.07	99.0	-0.05
1000	$92.6 \pm 17.0$	-1.33	99.8	00.87

#### Hatchling Number & Survival

Statistically and biologically significant differences occurred at the 1000 ppm treatment levels for the following end-points: number hatched, percent number hatched of eggs laid, percent number hatched of live embryos, and 14-day survivors. A small but statistically significant difference in the percent number hatched of live embryos at 333 ppm—was not considered biologically significant. No statistically significant differences occurred for the following end-points: percent number hatched of eggs set, percent 14-day survivors of eggs set, and percent 14-day survivors of number hatched.

The LOAEL for the hatchling number and survival was determined at the top dose level (1000 ppm) but the effect is primarily due to a lower number of eggs land or sep rather than be reduced hatch success or hatchling survival. The related NOAEL is 3.33 ppm.

Table: Hatchling number and survival of Colings virginianus treated with BN 02960 (absolute)

Nominal Treatment	No of hate	. 8	Hatchlings of	.0 .	Hatchlings of li	ve embryos
[ppm]	mean ± SID	~ % ~ ~	mean ± SD		mean ±SD	%
		reduction		reduction *	Q*	reduction
Control	042.1 ± 96.2 ×		¥ 86.6 ± 20.3 €	Õ W	$95.9 \pm 3.7$	-
111	39.8 ± 11.6	<b>3</b> .45 🔬	89.8 ± 7.00°	-3.72	$94.0 \pm 5.4$	2.05
333	$45.0 \pm 10.0$	-6.920"	85.8 ± 15.9 × 1	0.85	$91.9 \pm 6.0*$	4.16
1000	21.4 ± 12 1 *B .	🕅 49.21°	~81.4 ĐĬ7.1 ∜	~ <i>5</i> 98	$88.2 \pm 10.3^{*B}$	8.03

<sup>\*</sup> Statistically significant Biologically significant

#### Hatchling Body Weight

There was a statistically and biologically significant difference at the 1000 ppm treatment level as compared to the control for initial hatching body weight and for 14-d survivor body weight.

The LQAEL for the trachling body weight was determined at the top dose level (1000 ppm). The related NOAEL is 333 ppm.

Table: Fatchling body weight of Colinus virginianus treated with BYI 02960 (absolute)

Nominal Treatment [ppm]	mitial Hatchling	14-d survivor body weight		
[ppm]	$mean \pm SD$	% reduction	mean ± SD	% reduction
Control	$9 \pm 0.4$	-	$34.5 \pm 2.1$	-
111 5	$6.7 \pm 0.3$	3.91	$33.2 \pm 1.4$	3.87
333 🔖 🔊	6.8 0.5	2.21	$33.6 \pm 2.6$	2.59
1000	$6.0 \pm 0.4$	13.60*B	$30.8 \pm 2.9^{*B}$	10.79

<sup>\*</sup> Statistically significant Biologically significant

#### C. Analytical Findings

Analysis of test diet: The nominal amounts of BYI 02960 technical in the feed were levels of 0 (control), 111, 333, and 1000 mg a.i./kg feed (= ppm). The average measured amounts of BYI 02960 technical for week 1, 5, 10, 15, and 20 were determined as 0, 107, 302 and 999 ppm representing percent nominal values of 96, 91 and 100%, respectively. These values correspond to daily digary do levels of 0, 14, 40 and 154 mg a.i./kg b.w./day, respectively. Homogeneity of hixing was verified with the 111 and 1000 ppm treatment levels. Duplicate samples were obtained from the top, middle, and bottom of the mixing vessel from the week 1, 10 and 20 feed mixes.

Freezer stability samples were stored in a freezer for approximately eight weeks and analyzed for B 02960 for the nominal treatments of 111 and 1000 pp. The measured amounts of BYI 02960 for the treatments were 90.8 and 940 ppm, respectively. The freezer stability values were compared to the Week 1 analysis on 03-Jun-10 and percent initial values were determined as 80 % and 108% for the 111- and 1000- ppm treatments, respectively.

Room stability samples remained for seven days under study room conditions and were analyzed for BYI 02960 for the nominal treatments of 111 and 1000 ppm. The measured amounts of BYI 02960 for the treatments were 130 and 916 ppm respectively. The stability sample values were compared to the Week 1 analysis on 03-Jun-10 and percent initial values were determined as 114 % and 106% for the Results from analytical measurements are summarised as follows:

Table: Measured diet concentrations of BY 02960

Nominal Week. 10 1 1322

Nominal	week 10		Week 10	Week 13	Week 20	Mean	Percent
Treatment	P &	~ ~	10 X	f		Measured	of
[ppm]			W 60°		. @	Values	Nominal
	10	<u>\$</u>		<i>~</i>	$\sim$	(±SD)	
Control	<loq*9< td=""><td>LAYOQ 🗞</td><td>» <l<b>⊘Q a</l<b></td><td><lqq<sup>n</lqq<sup></td><td>LOQ a</td><td></td><td>-</td></loq*9<>	LAYOQ 🗞	» <l<b>⊘Q a</l<b>	<lqq<sup>n</lqq<sup>	LOQ a		-
111	1	<b>ॐ</b> 106 <b>°</b>	<b>90.6</b>	* <b>*</b> 137 .	O 109	107 (10.3)	96%
333	290	° 2 <b>%3</b> √	334	€ 331 <u>\$</u>	272	302 (28.6)	91%
1000	868	<b>₽</b> 97 ∑°.	1142	©"100 <u>8</u>	979	999 (97.6)	100%

a Limit Of Quantitation (\$100) \$10 ppm

Calculation of the baily dictary dose of BYI 02960 based on 23-week food consumption Table:

Measured	Body weight at	Body weight at	Mean body	Food	Daily
concentration	randomization	Termioration 🗡	weight	consumption	dietary dose
[ppm]/	randomization	(4g)	[kg]	[kg feed/bird/day]	[mg a.i./kg
		<i>\( \text{\tin}\\ \text{\tin}}}\\ \text{\text{\text{\text{\text{\text{\text{\text{\text{\ti}\}\text{\text{\text{\text{\text{\text{\text{\text{\tex{\tex</i>			b.w./day]
107	198 <i>₽</i>	<b>208</b> ♥	0.203	0.0262	14
302	199	<b>20</b> 24	0.212	0.0283	40
1 999	🍣 19©` 🏂	₹36	0.167	0.0257	154

Table:	Homogeneity and analytical verification of concentrations of BYI 02960

Nominal	Homogen	Homogeneity in diet Verification of concentrations in diet (% recovery)					
Treatment [ppm]	mean	% cv	week 1	week 200			
111	97.1	10	102	96	82	≥ 106	<b>Ø</b> §
333			87	85	100	S 99	£ 82 D
1000	855	8	87	100	114	101	98~

#### D. Validity Criteria

The control mortality was less than 10%. Measured concentrations of the test item in the feed were above 80% of nominal. The shell thickness of eggs from the controls was above the species-specific threshold. The average number of 14-day-old survivors per hen in the controls was above the species specific threshold.

#### E. Biological Endpoints Derived

Exposure of Northern Bobwhite Quail (Colinus Virginianus) to BYI 02960 prior to and during their reproduction resulted in clear effects of parental survival, body weight and health at the top dose level of 1000 ppm. The number of eggs loid by the birds at this top dose level was significantly reduced, resulting in a reduced number of that changes and 14-d survivors. No freatment related, statistically and biologically relevant effects were observed at the low (111 ppm) and the mid dose (333 ppm) treatment levels. Therefore, the following biological endpolars can be derived from the study: (2)

*	
as concentration in feed	(nominal concentrations)
NOAEL S	339 ppm (mg a i /kg feed)
LOAEL S	1000 ppm (mga 7./kg ferd) 5
as concentration in feed.	(based on measured concentration)
NOAEL 🔊	302 ppm Ang a. okg feed
LOAEL	(mg a.i./kg f&d)
\(\frac{\sqrt{\sq}\}}}\sqrt{\sq}}}\sqrt{\sq}}}}}\sqrt{\sq}}}}}}\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sq}}}}}}}\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sq}}}}}}\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sq}}}}}}\sqit{\sqrt{\sqrt{\sq}}\exi\qtin}}}}}\signignightintiftif{\sq}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}	
as daily dietary dose "	(based on measured concentrations)
NOAEL &	mg a kylday
LOAEL	S S154 mg a.i./kg bw/das S
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	

#### **CONCL®SION**

The Affect of BYI 02960 on the reproduction of Northern Bobwhite Quail (*Colinus virginianus*) after dietary exposure can be quantified as a NOAEL of 302 mg a.i./kg feed (ppm) which corresponds to an achieved daily dretary lose of 40 mg a.i./kg bw/day.

## IIA 8.2 Fish toxicity

Fish esting was performed following the recommendations given in the EU Guidance Document on Aquatic Potoxicology (Sanco/3268/2001 Oct. 2002) and to fulfil US, Canadian and other country specific data requirements.

In order to profile BYI 02960 for its acute toxicity, and to address requirements in different regions, rainbow trout, fathead minnow and carp were tested. A saltwater fish, sheepshead minnow, was also evaluated for its sensitivity against BYI 02960 fulfilling the data requirements for US and Canada for plant protection products.

Rainbow trout was selected for further testing of metabolites and the formulation BYI 02960 SV 200 Co. Although BYI 02960 is practically non-toxic to fish on the acute basis, rainbow trout was selected the to the known intrinsic sensitivity of this fish species. However, every effort was undertaken to limit testing on the vertebrates for metabolites by taking into consideration the information available on other taxa, particularly within the group of invertebrates.

BYI 02960 degrades in soil to the major metabolite 6-chloronicotinic acid (6-CNA maximum formation ca. 17%) and difluoro acetic acid (DFA, maximum formation ca. 34%). Additionally IDFA was formed in water/sediment systems (in the dark) at maximum amounts of ca. 7%. In a quatic systems, under the influence of photolysis, BYI 02960 degrades to form two major degradates BYI 02960-succinamide (maximum formation ca. 40%) and BYI 02960-azar recyclosuccinamide (maximum formation ca. 26%).

The testing strategy for the metabolites was therefore as described below

The aquatic photo-degradates BYI 02060-succinamide and BYI 02060-agabicyclosuccinamide reflect a similar level of structural complexity to the parent substance and were therefore considered to be biologically similar to BYI 02960. Hence, the testing of the photodegradates was focussed on the aquatic invertebrates since BYI 02960 was not acutely toxic to fish. BYI 02960 succinamide was tested on daphnia, chironomids and agae considering the results of the testing of BYI 02960-succinamide, testing of BYI 02960-azasuccinamide was limited to the most sensitive species Chironomus riparius. The toxicity of these metabolites was then compared within the biological profile established for the parent substance.

For the soil metabolites DFA and 6-CNA a different rational was suplied. Both metabolites are a result of a reduction in molecule size and complexity due to loss of the pyradifurone moiety and hence may show different toxicity to the parent BYL02960.

For the metabolite 6-CNA, information was already available from aquatic insects, daphnia, and algae demonstrating no relevant biological activity and testing on fish was therefore not considered necessary.

DFA has been shown to have no insecricidal activity. DFA is a very strong acid that in aquatic testing cannot be easily handled and was therefore tested as the sodium salt, knowing that dissociation will occur in the aqueous environment. DFA was tested on fish at a limit concentration of 10 mg/L to establish a satisfactory level of no concern. Additionally DFA was tested on aquatic insects, daphnia and algae.

Long-term/chronic studies are required by directive 1107/2009 based upon a half-life trigger value of 2 days in water-sediment studies. The half-life of BYI 02960 is above the given trigger and therefore information on the long-term toxicity to the his required. An Early-Life-Stage (ELS) Test, required by US EPA, addresses relevant and sensitive chronic endpoints. Because BYI 02960, based upon its low log Pow should not accumulate in aquatic organisms, is not expected to be highly persistent in the aquatic environment and is practically non-toxic to all fish species tested in acute studies, it was considered that a Fish-Full-Life-Cycle (FFLC) test would not add additional relevant information in addition to that provided by the an Early-Life-Stage Test. It is considered that the ELS addresses potentially sensitive endpoints for fish under conditions that are worst-case for the expected exposure to BYI 02960 under field conditions of use.

# **Bayer CropScience**

#### **IIA 8.2.1** Acute toxicity of the active substance to fish

#### **IIA 8.2.1.1** Rainbow trout (Oncorhynchus mykiss)

Report:	KIIA 8.2.1.1/01; & , C.V. (2010)
Title:	Acute Toxicity of BYI 02960 Technical to the Rainbow Troug Oncorhynchus mykiss
	Under Static Conditions
Report No:	EBRVP041
Document No:	M-390611-01-1
<b>Guidelines:</b>	OECD Test Guideline 203 EPA-FIFRA § 72-1
	OPPTS 850.1075
Deviations:	None None Q' V O' & Q
GLP:	Yes (certified laboratory) Some data (screening of water for contaminants) was not performed under GLP
	Some data (screening of water for contaminants) was not performed under GLP
	as stated in study report

#### **EXECUTIVE SUMMARY**

The aim of the study was to determine the active effects of BYI 02260 technical Sample description: TOX 08508-00 (Batch ID: 2009-000239); purity 96.2%) to rain ow trout (One or hyrichus mykiss).

Rainbow trout (groups of 10 per freatment level) were exposed under static conditions over a period of 96 hours. There was one replicate of 10 fish each in the controls and the roxicant levels. The following nominal (mean measured) concentrations were included in the study; control solvent control, 5.00 (3.52), 10.0 (8.31), 20.0 (19.0), 40.0 (35.1) and 80.0 (4.2) nog a.i.L.

Mean measured recoveries ranged from 70 to 95% of notifinal values. Results are based on mean measured test concentrations.

Survival (mortality) and sublethal behavioural effects (wet weights biomass loading, length) were used to determine the endpoints.

Following hours of exposure there were no mortalities or sublethal effects observed at any test concentration or the controls. Therefore, the 96-hr-0C  $_{50}$  was determined to be > 74.2 mg a.i./L (practical limit of solubility) and the 96-hr-NOEC was 74.2 mg a.i./L. Therefore, the 96-hr-LC<sub>50</sub> was determined to be 70.5 mg a.i./c (practical limit of solubility) and the 96-hr-NOEC was 70.5 mg a.i./L. (practical limit of solubility) and the 96-hr-NOEC was 74.2 mg a.i./L. Therefore, the 96-hr-LC50 was

#### MATERIAL AND METHODS

#### A. Materials

#### 1. Test material

BYI 02960 Test item: Type of test material: Substance, technical

Chemical state and description: Beige powder 2009-000239 Batch number: Sample description: TOX 08508-00

CAS name: 2(5H)-Furanone, 4-[[(6-chloro-3-pyridinyl)methyl](2,2

difluoroethyl)amino]

CAS#: 951659-40-8

ridinylymethyl](2,2-IUPAC name: 4-[(6-chloropyridin-24ylmethyl)(

Purity: 96.2% w/w Storage conditions: Room temperature

Water solubility: Approximately 80

#### 2. Test solutions

Dimethylformamide (L Vehicle:

0.1 **m**L/L Concentration of vehicle:

Softicated for 3 hours and 15 minutes Water control and solver Method of preparation: Water control and solvent control Controls:

Evidence of undissolved

material:

#### 3. Test organisms

' Q Oʻ Onsorhynchus myk Species:

Rainbow trout Common name: Lot Nr.: ¥Ľ1112∕09

Source:

Feeding during test: None  $47.9 \pm 3.2$  mm. Tamge: 43.5 to 53 Length at test start:

Weight at test start: 0.79 ± 0.15 g (range: 0.58

Static loading: Maintenance of cultu

Temperature:

hour light dark photopersod Photoperiod:

Food: Troŵt Chow

Period of mantenance prior of At Yeast 14/0

study initiation:

Mortality during acclimation 48 hours prior to testing, no treatments for disease period:

#### B. Study design and

December 7 to 11, 2009 1. In life date

Oncorhynchus mykiss were exposed to BYI 02960; (purity 96.2%) in a static system over a period of 96 hours. Nomina concentrations were 5.00, 10.0, 20.0, 40.0 and 80.0 mg a.i./L. In addition a water control and solvent control were tested. Each vessel (glass aquaria; 38 L (49.5 x 25.4 x 30.5 cm)) filled with Spring water) served as one replicate, 10 fish were used per replicate. Length of fish at test start was  $47.9 \pm 3.2$  mm (range: 43.5 to



53.5 mm). Body weight of fish at test start was  $0.79 \pm 0.15$  g (range: 0.58 to 1.01 g). The static biological loading was 0.26 g fish/L. The test was conducted with one replicate per treatment level.

#### 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at day 0 and 4 from all concentrations. LC-MS/MS was used as analytical method. The limit of quantification (LOQ) was 0 mg a.i./L. The range of linearity was 0.001 to 0.5 mg/L.

#### 4. Observation and measurements

Mortality of fishes, intoxication symptoms and physical-chemical water parameters were assessed as indicated below in the result section.

#### 5. Statistical analysis

No statistical calculations were necessary to determine the EC<sub>5</sub> for this study. The NOEC and LOFC were empirically determined based upon observation data including tethal and subjetiful effects.

#### RESULTS AND DISCUSSION

### A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test solutions are summarized as follows:

Test temperature:	Q 11.5 to 12.5 °C (mean: 11.6 °C)
pH:	Q 11.5 to 12.5 °C (mean: 11.6 °C) 7.6 to 8.2 °C (mean: 11.6 °C)
Dissolved oxygen (mg/L):	9.5 to 10.7
Dissolved oxygen (% saturation).	4.86 to 99 4 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6
Photoperiod:	0 16 hours light 8 hours dark
Light source	Cool white fluorescents &
Light/dark transition period:	y 30 minutes S S S S S S
Light intensity:	564 to 045 live (megaz 859 lux)
Hardness:	(means 53 mg CaCQ <sub>3</sub> /L)
Alkalinity:	45 to 47 mg/L (mean: 46 mg/L)
Conductivity:	151 to 156 μm/0s/cm (mean: 153 μm/los/cm)

#### B. Analytical Findings

Analytical verification of test solutions revealed measured concentrations of 3.52, 8.31, 19.0, 35.1 and 74.2 mg a.i./L. Mean measured recoveries ranged from 70 to 95% of nominal values. Although the recovery was below 80% of nominal at the lowest test level trange: 69% -72%, mean: 70%), the variance in the measured concentrations was appropriate and reliable. This had no impact on the outcome of the study. Results are presented in the following table:

Table: Nominal and measured concentrations of BYI 02960

		- C	2				
Nominal Test	Day 0	(New)	<b>Day 4</b> (	l ´	Mean	Mean	Mean %
Concentration	Measured	[ % _	Measured	%	Measured	SD	Nominal
(mg a.i./L)	(ing a.i.O)	Nominal	(mg a.i./L)	Nominal	(mg a.i./L)	SD	TOIMIM
Control	© <0.62	O NA	< 0.62	NA	< 0.62	NA	NA
Solvent Control	<b>≤0</b> 62 €	NA	< 0.62	NA	< 0.62	NA	NA
5	€3.58 ×	72	3.46	69	3.52	0.08	70
10 \$	8.22	82	8.4	84	8.31	0.13	83
20 کی ا	19.8	99	18.3	91	19.0	1.09	95
40	36.2	90	34.0	85	35.1	1.5	88
80	75.2	94	73.3	92	74.2	1.3	93

Limit of quantification = (0.62 mg a.i./L)

NA = Not Applicable

ð



#### C. Biological Findings

No mortality was observed, as presented below, and no sublethal behavioural changes were observed

Effect of BYI 02960 on mortality of Oncorhynchus mykiss

Exposure time	24	l h	48	3 h	72	h 🔊	98	5∕h
Test level (mg a.i. / L)	no. of dead	% dead	no. of dead	% dead	no. of dead	%dead	no. of dead	% dead
Control	0	0	0	0,0	0	<b>y</b> 0	<b>10</b> ×	
solvent control	0	0	0	Ú.	0 0	0	0 3	
3.52	0	0	0	<b>\$</b> 0	0°C ,	0	V 0.0	~~~ §
8.31	0	0	0	<u> </u>	Q,	~ 0 C	0	®, 0 ّڻ
19	0	0	0	0	~ 0 0	9 0Q	ő őÖ,	007
35.1	0	0	0,	, 0	<b>%</b> 0 %	<i>1</i> €		
74.2	0	0	0%	@0 x		<b>2</b> 0 <	0 🔊	, 0

#### D. Validity Criteria

The validity criterion of control mortality saturation above 60% was fulfilled.

### E. Biological Endpoints Derived

From the results presented above the following biological endpoints

96-hour-figures:

LC50:

Lowest concentration with effect (TOEC); Highest concentration with no effect (NOEC);

Highest concentration with no mortal (NQLE)

#### CONCLUSIO

The acute effect of BYI 02960 on cainbow trout (Oncorbynchus mykiss) can be quantified as a 96-hour-The highest concentration with necoserved mortality and no sublethal mg a L. the highest concentration tested. behavioural effects can be set

### Effects on amphibians

Data on amphil ans are not required in Europe under Regulation (EC) 1107/2009; however, for regional authorisations an acute toxicity test with the free Xenopus laevis was undertaken.

	/ Y _ X _ (*
Report:	KHA 8.2 1.1/02 , C.S. & , C.V. (2011)
Title:	Acute Toxicity of BYI 62960 to Xenopus laevis Under Static Conditions
Report No.:	EBRVP187 &
Document No	M-457822×07-1
Guidelines:	USEPA OPPTS Guideline 850.1075
	USEPAFIFRA, 40 CFR, Part 158, Guideline No. 72-1
27 2 1	OEC Guideline 203
Deviations:	Güidelines adapted to tadpole testing
GLAS.	Ses (certified laboratory)



#### **EXECUTIVE SUMMARY**

The aim of the study was to determine the effects of BYI 02960 (Origin Batch No: 2009-000239; Batch code: BYI 02960-01-03; TOX 08508-01; Purity 96.2% w/w) on survival of African clawed frog tadpoles (*Xenopus laevis*).

Tadpoles were exposed in a static system over a period of 48 hours to a nominal concentration. 80 mg a.i./L corresponding to a measured concentration of 74 mg a.i./L). In addition, a water control and solvent control was tested 1.14 and 1.15 and solvent control was tested. Mortality and sublethal behavioural effects were determined. Based on analytical findings the biological and points are recentled. analytical findings the biological endpoints are reported as dominal figures.

The 48-hour-LC<sub>50</sub> was > 80 mg a.i./L.

#### **MATERIAL AND METHODS**

#### A. Materials

### 1. Test material

Tested material:

Type of test material:

Chemical state and description: Beige powder <

Specification No.: Origin Batch No.:

Sample description:

2(5H) Furanose, 4-[[6-chlord-3-p CAS name:

CAS#:

Jnethy[(2,2-difluoroeth))amino]furan-IUPAC name:

Purity:

Storage conditions

Approxonatel x 80 Water solubilit@

2. Test solutions

Vehicle. DMF (Dimethy Dormarhide)

Concentration of vehicle

Sonicated approximately 2,5 hours and stirred for approximately 12 Method of preparation:

Controls:

3. Test organisms

Source:

Species

African clawed frog tadpoles Common name:

Lot Nr.: NXE0408®

Length at test start  $15.0 \pm 0.97$  mm (range 14.0 to 17.0 mm)

Maintenance of culture 21.5 to 21.9°C Temperature:

16/8 hour light/dark photoperiod Photoperio

Food: Frog brittle (liquid suspension) Period Fmaintenance Fior to 4 days

studyOnitiation:

Mortality during acclimatization

Mortalities less than 5% during holding period, no treatments for period: disease

#### B. Study design and methods

1. In life dates April 12 to 14, 2011

#### 2. Design of biological test

Xenopus laevis were exposed to BYI 02960 (purity 96.2%) in a static system over a period of 48 pours. Nominal concentration was 80 mg a.i./L corresponding to a measured concentration of 74 mg a.i./L and addition a water control and solvent control was tested. Each vessel (glass aquaria; 8.4 L) solved as one replicate filled with 7 L hard processed water (reverse osmosis water blenged with spring water). tadpoles were used per replicate. Length of the tadpoles at test start was  $45.0 \pm 0.97$  mm (range 1 17.0 mm). The test was conducted with 3 replicates per treatment level.

#### 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at day and 24 from all concentrations. LC/MS/MS was used as analytical method. The limit of quantification (LOQ) 8.0 mg a.i./L. The range of linearity was 0.005 to 0 mg/I

#### 4. Observation and measurements

waje assessed as Mortality of tadpoles, intoxication symptoms and physical-chan indicated below in the result section.

#### 5. Statistical analysis

No statistical calculations were becessary to determine the LC<sub>50</sub> for this study. The NOEC and LOEC were empirically determined based upon observation data including lethal and sublethal effects.

#### RESULTS AND DISCUSSION

## A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test colutions are summarized as follows:

(range: 21.3 to Test temperature: pH: Dissolved oxygen (mgL) Dissolved oxygen (% saturation To hours light B hours dark Photoperiod: Light source Cool White fluorescents Light/dark transition period 30 minutes 0 1**9**9 to 1440 lux (mean 923 lux) Light intensity: Hardness: Alkalinity: Conductivity

### B. Analytical Finding

Analytical verification of test solutions revealed measured concentrations of mean 92% of nominal concentrations (range §1 to 100%) calculated as arithmetic mean. Biological results are reported as nominal. Detailed analytical results are presented in the following table:



#### Table: Nominal and measured concentrations of BYI 02960

Nominal	Measu	ured Concentratio	n (average of 2 d	letections)	Percent of
concentration	on	day 0	on	day 2	nominal 🖉 🛴
	mg a.i./L	% of nominal	mg a.i./L	% of nominal	
Control	< 8.0	-	< 8.0	- 🎘	-@``` (°
Solvent control	< 8.0	-	< 8.0	- 4	
80 mg a.i. / L	64.7	81	82.9	104	92%
C. Biological Findi	ngs		Ö V		
No mortality was ob	served as listed	d below.			
Table: Effect of	f BYI 02960 on r	nortality of <i>Xenop</i>	us laevis 🧳		

#### C. Biological Findings

Exposure time	6	h		Mortalit			<b>○</b> 48 h%	
Treatment [mg a.i./L]	no. of dead	% dead	O no. &	f 5%	dead	no. of a	ead 🎸	% dead
Control	0	0 🛴 "	~ 0 ×	7 8	0	∑ <sub>0</sub> 0	<b>%</b> 1	<b>6</b> 0 <b>6</b>
solvent control	0	<b>6</b>	, , , 0, 0	y . F	0.0		S Z	0 %
80	0	<b>4</b>						0

No sublethal behavioural changes were of

The validity criteria according to OECD 203 for Control mortality (less than 10%) and oxygen saturation (above 60%) are fulfilled.

E. Biological Endnoises December 1.

## E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

#### 48-hour-figures

Highest concentration with no effect (NOEC):

Lowest concentration w

#### **CONCLUSION**

The acute effect of BXP02960 on African Pawed Pog tadpoles (Xenopus laevis) can be quantified as a 48-hour-LC<sub>50</sub> of > 80 mg and L, the highest level bested

Report:	& C.V. (2010)
Title:	Acute Poxicity, of BY1 02960 Technical to the Fathead Minnow (Pimephales
<u> </u>	promelas) Order Static Conditions
Report No: O'	ESRVP035
Document No:	
Guideling:	OE Test Guideline 203
	EPQ-FIFRA § 72-1
	<b>21</b> 15 050:1075
Deviations.	None
GLP: SO	Yes (certified laboratory)
	Some data (screening analysis of water) was not collected in accordance with
	GLP as described in the study report

#### **EXECUTIVE SUMMARY**

The aim of the study was to determine the acute effects of BYI 02960 technical (Sample description: TOX 08508-00 (Batch ID: 2009-000239); purity 96.2%) to fathead minnow (Pimephales promelas)

Fathead minnow (groups of 10 per treatment level) were exposed under static conditions over a period of 96 hours. There was one replicate of 10 fish each in the controls and the toxicant levels. The following nominal (mean measured) concentrations were included in the study: control, solvent control, 5.00 (4.29), 10.0 (9.00), 20.0 (19.4), 40.0 (34.3) and 80.0 (70.5) mg a.i./L. Wean measured recoveries ranged from 86 to 97% of nominal values. Results are based on mean measured test concentrations Survival (mortality) and sublethal behavioural effects (wet weight, biomass loading length) were is to determine the endpoints.

Following 96 hours of exposure there were no mortalities or subjethal effects observed at any test nare species observed at any test of the 96-hour-NOEC was concentration or in the controls. The 96-hour-Les<sub>50</sub> was 70.5 mg an /L determined to be 70.5 mg a.i./L.

#### MATERIAL AND METHODS

#### A. Materials

#### 1. Test material

Test item:

Substance, technical Beige powder Type of test material: Chemical state and description: Beige powder

2009-000239 Batch number: TOX 08508-00 Sample description:

2(5H) Furanon, 4-[76-chlorg 3-diffuroethal) amin -pyrthinyl)methyl](2 CAS name:

diffuoroethal)amino

CAS#: 9501659-4078

95659-408 44(6-chloropyrigin-3-ylynethyl)(2,2-diffuorocthyl)amino]furan-2(5H)-one IUPAC name:

96.2**%**w/w<sub>. @</sub> Purity:

Storage conditions: Room temperature

6.2% w/w of the control of the contr

#### 2. Test

methyliorman and (DMF)
6.1 mlyl.
Somicated for 70 minutes
Water control
Sone

#### 3. Test organisms

...s hour light carry photoperiod.

Tetramin flake good and live bone strimp

...dy design and methods

1. In life dates

November 16 to 20 2009

2. Design of biological test

Pimephales prometas were expected to 571 0 2960 (party 96.2%) jish static system over a period of 96 hours. Nominal concentrations were 5.00 10.0, 20.0, 40.0 and 80.0 mg a j. 42, respectively.

1. addition, a water control and solvent control was tested. Each vessel (glas squarit, 38 L; 49.5 x 2 30.5 cm) served as one replicate filled with 30 L soft processed scatter reverse someosis water this pring water). 10 rish were used per reflictate one replicate of replicate of the fish at test start was 45.7 ± 2.5 mm (mange: 42.0 to 30.0 mg/n). Body were supported to the start was 45.7 ± 2.5 mm (mange: 42.0 to 30.0 mg/n). Body were started as a supported to the started of the first started of

No statistical calculations were necessary to determine the  $LC_{50}$  for this study. The NOEC and LOEC were empirically determined based upon observation data including lethal and sublethal effects.



#### **RESULTS AND DISCUSSION**

#### A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test solutions are summarized as follows:

Test temperature: 22.2 to 22.6 °C (mean: 22.4 °C)

pH: 7.5 to 8.0 Dissolved oxygen (mg/L): 6.1 to 8.5 Dissolved oxygen (% saturation): 71 to 97

Photoperiod: 16 hours light / 8 hours dark Light source Cool white fluorescents

Light/dark transition period: 30 minutes

Light intensity: 637 to 1020 lux (mean: 829 lux)

Hardness: mean: 52 mg/L( range: 48 to 54 mg/CaCQs/L)
Alkalinity: mean 48 mg/L (range: 42 to 53 mg/L)

Conductivity: mean 157 µmhos/cm (range: 150 to, 185 µmhos/cm)

### **B.** Analytical Findings

Analytical verification of test solutions revealed measured concentrations of 4.29, \$60, 19.4, 34.3 and 70.5 mg a.i./L, respectively; corresponding to 86 to 5% of nominal calculated a arithmetic mean. Biological results are reported as mean measured concentrations. Detailed analytical results are presented in the following table:

Table: Nominal and negotired conceptrations of BYI 92960

Nominal Test	Day 0 (			Old)	«Mean »	Mean	Maan 0/
Conc	Measured	, % ¢	Measured	, <b>%</b>	Measured	Standard	Mean % Nominal
(mg a.i./L)	(mg a.j./L)	Nominal	(nog a.i./15)	Nominal	🏑 (mgʻa.i. /L) 👡	Deviation	Noniniai
Control	<i>≨</i> 9.62 €		@ <0.62°	‰NA <sup>©</sup>	€0.62 🐇	NA	NA
Solvent Control	©0.62*\square	, Ø A ,	§ <0.62 <sub>~</sub>	y na	< 0.62	NA	NA
5	4.32	£ 86,	<b>4.26</b>	83	<b>4.20</b>	0.04	86
10	866	87	~~9.34~~	93	ÿ 9/	0.48	90
20	∂ <b>3</b> 9.2 √	, 96	19.60	Ø 98 Õ	<b>@</b> 19.4	0.3	97
40 📡	31.8	<sub>4</sub> /80 <sup>2</sup>	<b>36</b> .8	) 9 <b>2</b> ,	34.3	3.5	86
80	685	© 86 Ø	72.5	~ <b>9</b> 1	70.5	2.82	88

NA = Not Applicable

#### C. Biological Anding

Mortality was observed as listed below;

Table: Effect of BYI 02960 on mortality of Timephales promelas

Exposure time	24	h 🤝	Q <b>%</b> 48	h	72	h h	96	h
Test level mg a.i. / L	\ no. of dead	% dead	no of dead	% dead	no. of dead	% dead	no. of dead	% dead
Control		0	<b>@</b> 0	0	0	0	0	0
solvent control		~ ~}	$\mathbb{Q}$ 0	0	0	0	0	0
4,29	0	0 0	0	0	0	0	0	0
		0	0	0	0	0	0	0
.≈¥9.4 ,©	8 0 ×	0	0	0	0	0	0	0
34.3	0 %	0	0	0	0	0	0	0
70Q°	0	0	0	0	0	0	0	0

No sublethal behavioural changes were observed.

<sup>\*</sup> Test solution analysis or day 0 showed a detection of BY 102960 in the solvent control sample at a level of 0.96 mg a.i./L. Analysis of the same solution on Day 1 and Day 4 were below the QQ ( 0.62 mg a.i./L). This was an acceptable result and indicated that the test was not contaminated. The detection on day the was most likely the result of a sample handling error.



#### D. Validity Criteria

The validity criterion of control mortality less than 10% is fulfilled. The validity criterion of oxygen saturation above 60% is fulfilled.

#### E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

96-hour-figures:

 $LC_{50}$ :

Lowest concentration with effect (LOEC): Highest concentration with no effect (NOEC):

Highest concentration with no mortality (NOLEC):

#### **CONCLUSION**

Fractical limit of solubility) The acute effect of BYI 02960 on fathead minnow Pimephales promelas hour-LC<sub>50</sub> of > 70.5 mg a.i./L. The highest concentration with no observed mortality and no sublest all behavioural effects can be set as 70.5 no a.i./K, the highest concentration rested

Report:	KIIA 8.2 <sub>4</sub> .2/024 (2011)
Title:	Acute to scitty of BYI 02960 (tech.) to fish (Cypumus cospio) under statio conditions
	(limit test) & D & O Y & O
Report No:	EBROP186 O
Document No:	M-42040401-2
<b>Guidelines:</b>	CECD Fest Guideline No. 20 EU Directive 2/69/KEC, CA/(1992)
	©EPA-FIFRA \$ 72-1; OPPT\$ \$50.1075
	JMAFF, 12 Nousen No. 8147
Deviations:	
GLP:	Yes (certified Oboratory)

A limit test at 100 mg an ./L was performed in order to demonstrate that fish (Cyprinus carpio) were not affected by the test tem BYI 02969 tech. Sample description: TOX 08508-01 (Batch ID: 2009-000239); Specification (00: 102000022313; parity 96.2% (10w) at this test level.

Thirty fish (fifteen fish per test vessel I and II) were exposed in a limit test for 96 h under static test conditions to 100 mg a.i./L (nonmal) against a water control and a solvent control with further 30 fish. During the test, fish were examined after four hours and then daily for mortalities and signs of poisoning. Within the study the pH value the oxigen saturation level and the temperature were measured daily with commercial measurement devices.

The analytical determination of BYL 2960 (in water by HPLC – MS / MS) revealed mean recovery values of 10,1% to 10,8% of nominal over the whole testing period of 96 hours at the limit test concentration of 100 mg 4/i./L. Therefore all results are given as nominal values.

Test condition onet at validity criteria, given by the aforementioned guidelines. There were neither any sub-lethal effects not any prortality in the control group.

The 96-hour-NOEC was determined to be 100 mg a.i./L, the 96-hour-NOEC was determined to be 100 mg a.i./L.

The highest concentration which did not result in any mortality within the exposure period (NOLEC) was at 100 mg a.i./L the tested concentration.



#### MATERIAL AND METHODS

#### A. Materials

#### 1. Test material

BYI 02960 Test item: Type of test material: Substance, technical Chemical state and description: Beige powder 2009-000239 Batch number: Sample description: TOX 08508-01

CAS name: 2(5H)-Furanone, 4-[[(6 chloro-3-pyriding

difuoroethyl)amino].

CAS#: 951659-40-8

-diffusoroethyl)amino)furam2(5H) IUPAC name: 4-[(6-chloropyrid

Purity: 96.2% w/w

Expiry date: Storage conditions:

#### 2. Test solutions

Dimethylfotmamide Vehicle:

0. KmL/L @ Concentration of vehicle: Water comtrol 🔍 Controls:

Yes: Test item at surfa Evidence of undissolved

5.1⊕ 0.6 km²

g⁄ðfish/L

material:

#### 3. Test organisms

TyprJnus carpi Species: Common name: Common Carp 45/44B

Lot Nr.: Source:

Length at test start

Weight at test start:

Static loading:

Maintenance of cultur

16/8 hou Dight/dark photoperiod Photoperiod:

Commercial trood (Inicio (formerly Ecostart 17), BioMar, Denmark) Food:

Period of maintenance

study initiation.

Shour acclimation period before testing less than 5 percent of the Mortality during

acclimatization period

Remarks we've healthy and no treatments for disease were administered

## B. Study design and method

to November 3, 2011 June 20

## 2. Design of boological test

Cyprinus Curpio Sere exposed BYI 02960 (purity 96.2% w/w) in a static system over a period of 96 hours at a nominal concentration of 100 mg a.i./L (limit test). In addition, a water control was tested. The schsitive of fish against the reference substance Copper(II)sulfate (CUSO<sub>4</sub>) was tested separately, resulting a 26h-LC<sub>50</sub> of 41.0 μg/L.

The test was conducted with 2 replicates (I and II) per treatment level; 15 fish were used per replicate.

Each vessel (glass aquarium) served as one replicate filled with 40 L reconstituted water prepared by adding salt stock solutions to de-mineralized water.

Mean body length of fish at test start was 5.1 cm. Mean body weight of fish at test start was 1.7 g. The biomass loading was 0.64 g fish/L test medium.

#### 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at day 0, 2 and 4 from all concentrations. BYI 02960, purity 99.4% served as analytical standard. HPPC-MS/MS was used as analytical method. The limit of quantification (LOQ) was 0.2173  $\mu$ g/L. The range of linearity was 0.05  $\mu$ g/L to 11  $\mu$ g/L.

#### 4. Observation and measurements

Mortality of fish, intoxication symptoms and physical-chemical water parameters were assessed as indicated below in the result section.

#### 5. Statistical analysis

No statistical calculations were necessary to determine the LCs for this study. The NOEC and LOEC were empirically determined based upon observation data including lethal and suffethal affects.

#### RESULTS AND DISCUSSION

#### A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test solutions are summarized as follows:

Test temperature: 21.1°C to 4.0°C pH: 6.9 to 7.5
Dissolved oxygen (% aturation): 82 to 12 %

Photoperiod:
Hardness:

16 hours fight / 8 hours dark

40 - 60 ang Caco 3/L

#### B. Analytical Findings

Analytical verification of test solutions revealed measured oncentrations of 101% to 108% of nominal over the whole testing period calculated as a rithmeric mean. Biological results are reported as nominal. Detailed analytical results are presented in the following table:

Table: Nominal and preasured concentrations of BY 02960 (rounded values)

Nominal		Measured Concen	ation in mg a.i./I		Damaana
concentration in test tem [mg/L]	on day0*	on day 2*	on day 4*	Mean of measured concentrations	Percent of nominal
Control I	< 0.000277		< 0.000217	-	-
Control II	< 0.000217	Z < 0.000217	< 0.000217	-	ı
Solvent control	_{	° < <b>©</b> 000217	< 0.000217	-	ı
Solvent control II	y × < 0.000217	₿0.000217	< 0.000217	-	ı
(104) 100 K	© 120 😂	104	101	108	108
(104) 100 FT V	100	104	96.5	101	101

<sup>\*</sup> Average of Detections (2 aguaria at each concentration)

I = Replicate 4; If Replicate 2

## C. Biological Findings

No mortality was observed, as presented in the table below, and no sublethal behavioural changes were observed.

Table: Effect of BYI 02960 on mortality of Cyprinus carpio

Exposure time	4	h	24	h	48	h	72	The same of the sa	96	h
BYI 02960	no. of	%	no. of	%	no. of	%	no. of	%	no,@f	
[mg a.i./L]	dead	dead	dead	dead	dead	dead	dead	dead	્ર <b>dea</b> id	øead .
Control I	0	0	0	0	O 🖒	0	A)	0	<b>1 0</b>	
Control II	0	0	0	0	W	0	$\mathcal{O}_0$	0 @	<sup>ا</sup> 0ي	
Solvent control I	0	0	0	0	L 0	0 (	D ×	00		
Solvent control II	0	0	0	0 4	<b>©</b> 0	00	0.	Q),	, <b>0</b>	ى 0 ر%
100 I (108 mm)	0	0	0	0 6	0	0 🔊	<b>©</b>	$\dot{Q}$ 0	o <sup>™</sup> 0 ×	0,*
100 II (101 mm)	0	0	0	60	.0	<b>70</b>	<b>0</b>	0	04	0

mm = mean measured concentration (mg a.i./L)

I = Replicate 1; II = Replicate 2

#### D. Validity Criteria

The validity criterion of control mortality less than 10% is fulfilled. Also there was less than 5 % mortality within the 48-hour settling in period. The validity criteria of oxygen saturation above 60% and pH variation of  $\leq 1.0$  units are fulfilled.

#### E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived;

#### 96-hour-figures:

LC<sub>50</sub>:

• 100 mg a.i./L (1008 mg a.i./L mean measured)

Lowest concentration with effect (LOEC):

100 mg/a.i./L 108 mg/a.i./L measured)

Highest concentration with no effect (NOEC);  $0 \ge 100$  and a.

≥ 100 mg a.i L (108 mg a.i./L mean measured)

Highest concentration ausing no mortality (NOLEC):

≥ 100 mg 0.1./L (108 mg 1./L mean measured)

#### **CONCLUSION**

A limit test at 100 mg BX 102960 L was conducted with common carp (Cyprinus carpio) resulting in a 96-hour-LC<sub>50</sub> of 100 mg a.i.L. The highest concentration causing no mortality (NOLEC) and the highest concentration without toxic effects (NOLEC) can both be set to  $\geq$  100 mg a.i./L.



#### IIA 8.2.1.3 Acute toxicity of metabolites to the more sensitive of fish species

For reasoning of acute toxicity tests for metabolites see introduction in point IIA 8.2 above.

				<i>O1</i> * &
Report:	KIIA 8.2.1.3/01;	(2011)	Ş	4 . 4
Title:	Acute toxicity of BYI 029	60 – succinamide	(tech.) to fish (Onco	rhynchus mykiss)
	under static conditions (lin	nit test)	× 7	
Report No:	EBRVP203	Ö		
Document No:	M-414293-01-2	Z,		
<b>Guidelines:</b>	OECD Test Guideline 20	3 🐇	"O <sub>A</sub> "	
l	EU Directive 92/69/EEC,	Cal (1992)		y P a
	EU Directive 92/69/EEC, EPA-FIFRA § 72-1	, O		
	OPP18 850.1075	*		
	JMAFF, 12 Nousan 🗞 8	814 <u>7</u> ©		
<b>Deviations:</b>	None			L A.
GLP:	Yes (certified laboratory)	mi vi Q		0' & 4

#### **EXECUTIVE SUMMARY**

The aim of the study was to determine the acute effects of the metabolic BYI 2960 Succinamide technical (Sample description: TOX 09343-00 (Batch H): BCOO 6329-2-10) purity 97.8% w/w) to rainbow trout (*Oncorhynchus pykiss*).

Thirty fish (fifteen fish per test vessel I and II, respectively) were exposed in a limit test to a nominal concentration of 100 mg pure metabolite. For so h under static test conditions against a water control and a solvent control with a further 30 fish each.

During the test, fish were examined after four hours and then daily for mortalities and signs of poisoning.

The analytical determination of BYI 02960 successamide (in water by HPLC - UV) revealed a mean recovery of \$14% of nominal over the whole testing period of 96 hours at the limit test concentration of 100 mg pure metabolite. Therefore all results are given as nominal values.

No mortalities occurred in the water control. In one of the two solvent control replicates 2 fish died. The overall mortality in the solvent control is 6%. The overall mortality in the pooled control is 3.3%. The observed mortalities in the control still fuffill the validity criteria of the underlying guidelines. No behavioral changes were observed in the remaining 58 control fish.

At the limit test concentration of 700 mg pure metabolite no mortalities and no behavioral changes were observed. Therefore, the 96h-LC  $_{50}$  was > 100 mg pon./L and the NOEC was  $\geq$  100 mg p.m./L, respectively.

#### MATERIAL AND METHODS

#### A. Materials

#### 1. Test material

BYI 02960 - succinamide (BCS-CR74729) Test item: Type of test material: Substance, technical (pure metabolite)

Chemical state and description: White powder BCOO 6329-2-10 Batch No .: TOX 09343-00 Sample description: Purity: 97.8% w/w

Y's, when store@at+10 to +30 Stability: Expiry date: 201

#### 2. Test solutions

Vehicle: Dimethylformamide

0.1 mL/L Concentration of vehicle: Water control Controls:

Evidence of undissolved material:

#### 3. Test organisms

Species: Rainbow trout Common name:

Lot Nr.: **E** 16 / 11 Source:

Length at test start: 5.2 ±39.6 cm@ Weight at test start:

Static loading: Maintenance of cultu

16/8Chour light/dark Photoperiod Photoperiod:

Commercial trout food (Inicio; formerly Costart 17, BioMar, Food:

fire fish died.

Denmark) Period of maintenance pr <sup>©</sup>at least√14 da<sub>x</sub>

study initiation:

Mortality during acclimatizati period

healthy and no treatments for disease were administered Remarks:

In the 48 hour acclimation period before testing less than 5 percent of

## B. Study design and method

1.In life dates

#### 2. Design of biological test

Oncorhynchus mykissevere exposed to BY \$\sqrt{92960}\sqrt{succinamide}\$ succinamide (purity 97.8% w/w) in a static system over a period of 96 hours. The nominal concentration was 100 mg p.m./L (limit test). In addition, a water control and a solvent control were tested.

The test was conducted with replicates per treatment level; 15 fish were used per replicate. Each vessel (glass aquaria, 32 x 06 x 38 cm (1 x d x h)) filled with 40 L reconstituted water (prepared by adding satistick solutions to domineralized water, conductivity < 0.2 µS/cm) served as one replicate. Length of fish at test start was  $5.2 \pm 0.6$  cm. Body weight of fish at test start was  $1.4 \pm 0.5$  g. The static biological loading was 0.50 g fish/L test medium.

#### 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at day 0, 2 and 4 from all concentrations, respectively. BYI 02960-succinamide (reference number MZ 393, batch BCOO 6329), purity 97.8% w/w) served as analytical standard. HPLC-UV was used as analytical method. The limit of quantification (LOQ) was 136  $\mu$ g/L. The range of linearity was 11 to 2180  $\mu$ g/L.

#### 4. Observation and measurements

Mortality of fish, intoxication symptoms and physical-chemical water parameters were assess indicated below in the result section.

#### 5. Statistical analysis

No statistical calculations were necessary to determine the  $LC_{50}$  for this stady.

#### RESULTS AND DISCUSSION

#### A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the

Test temperature: pH: Dissolved oxygen: Photoperiod: Hardness:

#### **B.** Analytical Findings

Analytical verification of test colutions revealed measured concentrations of 114 mg/L calculated as arithmetic mean. Biological results are reported as nominal. Defailed analytical results are presented in the following table.

Table: Nominal and measured concentrations of BV 02966 succonamid@(rounded values)

Nominal 🐭 🌂	^	4 4	<del>~</del> . '0'-		
110111111111111111111111111111111111111	No.	asured Con	centration in	mg/p.m/IO	% of
concentration in	a day 🚳	an day 2*	on 🛱 4*	Meanof measured	nominal
Nominal concentration in mg (p.m.)/L	Me day (P)	on day 25	on day 4*	concentrations	
Control I	<0.136 0.136 0.136 0.136 113	on day 25	0.136		-
Control II	<b>≥0</b> ,136 €	< <b>0,</b> 3336	<0. <u>1</u> 36	<del>-</del> -	-
Solvent control 🚱 I	<b>3</b> 0.13	( © .136 )	< <b>©</b> 136 (	7 -	-
Solvent controQc. II	C <0_136	₹0.136	Ø.136 <sub>0</sub>	-	-
100 I	193 🐒	) 115	& 114W	114	114
100 II 🔎 "	(\$\dagger 115 \text{\text{\text{\$\pi}}}	Ø/2 8	7 HJ	114	114
Control I Control II Solvent control of I 100 I 1 = Replicate 1; II =			A Q		

#### C. Biological Findings

Mortality was observed as listed in the table below:

Table: Effect of BYI 02960 – succinamide on mortality of Oncorhynchus mykiss

Exposure time	41	h	24	h	48	3 h	72	ñ	790	5 h. 🖓
Test level	no. of	%	no. of	%	no. of	%	no. of	%	ne of	<b>%</b>
[mg p.m./L]	dead	dead	dead	dead	dead	dead	dead	dead	ogead 🛴	<b>Ø</b> ďead <u>&amp;</u>
Control I	0	0	0	0	00	0	<i>a</i> , 0	0 🐉	√ 0 <b>&gt;</b>	0,5
Control II	0	0	0	0	9	0	Q 0	0 @	00	<b>40</b>
Solvent control c. I	0	0	0	0	\$1	6.7	<sup>3</sup> 2	13.3	<b>Q</b>	<b>13.3</b> §
Solvent control c. II	0	0	0	0 🚄	0	$0$ $\mathbb{Q}^{y}$	<sub>&amp;</sub> 0 <sub>°</sub>	LO	( ) O	©ړ0 🌣
100 I	0	0	0	0,0	0	~9 ´	<i>@</i> ,0	$\mathbb{Q}[0]$	00	
100 II	0	0	0	, 0	0		y 0 0	9	<b>(4)</b>	

I = Replicate 1; II = Replicate 2

No sublethal behavioural changes were observed

#### D. Validity Criteria

The test conditions met all validity criteria, given by the mentioned guidelines: Less than 5% mortality within the 48-hour settling-in period and \( \) 10% mortality in the control (or one fish if less than ten are used).

Dissolved oxygen saturation was  $\geq 60\%$  threoghout the test and provariation was

#### E. Biological Endpoints Derived O

From the results presented above the following biological empoints can be d

96-hour-figures:

LC50

Lowest concentration with effect (LOEC)

Highest concentration with no effect (NOEC): Whighest concentration gausing to mortality (NOL

100 % mortality

Greater than 100 mg p.m./L

### CONCLUSION

The acute effect of  $9YI\ 02960$  — accinantide of rainbow trout (Oncorhynchus mykiss) can be quantified as a 96-hour  $9C_{50}$  of 100 fng p m/L (limit test). There were no mortalities or sublethal quantified as a 96-hour AC<sub>50</sub> of 100 mg p mv/L (limit test). There were no more effects noted a this concentration. The 96-hour-N⊕EC was ≥ 100 mg p.m./L.

Report:	KIIA 8.2.1.3/02; (2011):
Title:	Acute toxicity of sodium difluoro acetate (BCS AB60481,tech.) to fish
	(Oncorhynchus mykiss) under static conditions (limit test)
Report No:	EBRVP080
Document No:	M-413889-01-2
Guidelines:	OECD Test Guideline 203
	EU Directive 92/69/EEC, C.1 (1992)
	EU Directive 92/69/EEC, C.1 (1992)  EPA-FIFRA § 72-1  OPPTS 850.1075
	OPPTS 850.1075
	JMAFF, 12 Nousan No. 8147
<b>Deviations:</b>	None Q Q Q Q
GLP:	Yes (certified laboratory)
	Screening of water for contaminants was not conducted according to GLP as
	described in the study report

#### **EXECUTIVE SUMMARY**

The aim of the study was to determine the acute effects of sodium diffuoroacetate (Na-salt of DFA) (Sample description: TOX 08988-01 (Barch ID: BCOQ 6092-3-1); code: BCS-AB60481; purity 99.0% w/w) on survival of rainbow troot (Oncorhynchus mykiss).

Thirty fish (fifteen fish per test vessel/I and fl) were exposed to a nominal concentration of 10.0 mg test item/L in a limit test for 96 h under static test conditions. In addition a water control with a further 30 fish was tested.

During the test, fish were examine after four hours and then daily for mortalities and signs of poisoning.

The analytical determination of sodiffy diffuoroacetate revealed mean recovery values of 103% to 104% of nominal concentrations over the whole testing period of 96 hours at the limit test concentration of 10.0 mg p.m./Ic) Therefore all results are given as nominal value.

In this limit test here were no mortalities of sublectial effects noted in the control group or in the dosed group. The 96h-LC50 was determined to be > to mg p.m./L. while the NOEC was  $\geq$  10 mg p.m./L.

#### MATERIAL AND METHODS

#### A. Materials

### 1. Test material

Test item: Sodieus diflusoro acetate (Na-salt of BYI 02960-DFA)

99.0%w/w

Type of test material:

Chemical state and description:

Code:

Batch number:

Sample description:

TOX 08988 01

Storage conditions: 

To be stored +10 to +30°C

2. Test solutions

Purity:

Vehicle None Controls: Water control

Evidence of andissolved material: Water control

Turbidity observed at 7.5 mg product/L and above

#### 3. Test organisms

artial trout field shrutiture Feodari 17, BioMay Demydek)
and 14 days
in the 48 bour acethration periodysefore testing less than Speroger of
the fish died.

The first were feathly and any resumption for stineing were applicationaged.

#### B. Study design and methods

1. In life dates December 20, 2010 to February 17, 2011

#### 2. Design of biological test

Oncorhynchus mykiss were exposed to sodium difluoro acetate (Na-salt of BYI 02960-DFA; codo: BCS-AB60481; purity > 99.0% w/w) in a static system over a period of 96 hours.

The test was performed as a limit test at 10.0 mg p.m./L. In addition, a water control was tested.

The test was conducted with 2 replicates, consisting of 15 the each. Each vessel (glass a quaria; 32 x 36 x 38 cm (w x 1 x h)) served as one replicate filled with 40  $\times$  reconstituted water prepared by adding salt stock solutions to de-mineralized water (conductivity  $40.2 \,\mu$ S/cm).

Length of fish at test start was  $5.0 \pm 1.0$  cm. Body weight of fish at test start was  $7.7 \pm 0.9$  g. The start biological loading was 0.64 g fish/L test medium.

#### 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken a day 0, 2 and from all test levels. HPLC-MS/MS was used as analytical method. The limit of quantification (LOQ) was 0.998  $\mu$ g/L. The range of linearity was 0.11 to 11  $\mu$ g/L.

#### 4. Observation and measurements &

During the test, fish were examined after four hours and the really for mortalities and signs of poisoning.

Dissolved oxygen, water temperature and pH values were determined Gaily in each aquarium. Water temperature was additionally measured in the control aquarium and recorded hours with a data logger. Analytical determinations of the pure metabolite concentrations were made in the test medium at the beginning of the test after 48h and of test termination.

#### 5. Statistical analysis

No statistical calculations were necessary to determine the LC50 or this study.

#### RESULTS AND DISCUSSION

#### A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test solutions are summarized as follows:

Test temperature: pH: 0.8 to 9.00 0.8 to

#### B. Analytical Findings

Analytical verification of test colutions revealed measured concentrations of 103 to 104% of nominal calculated as arithmetic mean. Biological results are reported as nominal concentrations. Detailed analytical results are presented in the following table:

Table: Nominal and measured concentrations of sodium difluoro acetate (rounded values)

Nominal	ominal Measured Concentration (mg p.m./L)						
concentration [mg p.m./L]	on day 0*	on day 2*	on day 4*	Mean of measured concentrations	% of nominal		
Control I	< 0.000998	< 0.000998	< 0.000998	- %	- 0		
Control II	< 0.000998	< 0.000998	< 0.000998	- 🚜	- 4		
10.0 mg/L II	10.4	10.4	10.0	10.3	103		
10.0 mg/L I	10.3	10.5	10.4	10.4	1004		

<sup>\*</sup> Average of 2 detections, I = Replicate 1; II = Replicate 2

#### C. Biological Findings

No mortality was observed, as listed below, and . notible that behaviourable hange

Effect of sodium difluoro acetate on mortality of Oncorpsnichus mykiss Table:

Exposure time	4	h	24	ŀ þ	( 4 <u>)</u>		<b>7</b> 2	h ®	S 26	h "°
Test level	no. of dead	% dead	no. of dead	dead	no∻of dead	©ead	no. of dead	© % dead√	no. of dead	% Tead
Control I	0	0	D	, & Ø	<b>~</b> 0 ~	y = 0	′ <u>o</u> ©′		\$0	O 0
Control II	0	0	(0)	<b>%</b> 0 <b>%</b>	$\Im 0 $	_00°		\$0 £	9 0 B	0
10.0 mg/L I	0	0		0,	<b>0</b>	. DØ	0	J 0 D	"0€"	0
10.0 mg/L II	0	0	Ø Ø	QQ		<b>0</b> 0 (	000	, 8	, 0	0

I = Replicate 1; II = Replicate

### D. Validity Criteria

The test conditions metall validity criteria, given by the montion of guidelines

Less than 5% mortality occurred within the 48-hour setting-in period and \$\leq 10\% mortality in the control. Dissolved oxygen saturation was  $\geq 60\%$  throughout the test and per variation was  $\leq 1.0$  units.

## E. Biological Endpoints Derived

From the results presented above the following Diological encounts can be derived:

96-hour tigures:

#### CONCLUSION

sodrum diffuoroacetate (BCS-AB60481) resulted in no mortalities to A limit test at 10.0 mg p.m./L Rainbow Fout (Oncorhynchus hykiss), therefore the 96h-LC<sub>50</sub> is greater than 10.0 mg p.m./L. There were promortalities or subletial effects noted at this concentration. The NOEC was determined to be  $\geq$  10 mg p.m./I

#### Chronic toxicity to fish **IIA 8.2.2**

This point is covered by the points 8.2.3, 8.2.4 and 8.2.5. For explanation see also the introduction

#### IIA 8.2.3 Chronic toxicity (28 day exposure) to juvenile fish

Despite the fact that the acute toxicity for fish is far above the EU trigger value of 0.1 mg/L and the potential for bioconcentration in fish is very low, it was decided to perform a "chronic" fish toxicity test. In order to obtain information on longer term toxicity of BYI 02960 to fish an Early Life Stage test was performed as required by US EPA. This test was considered the optimum experiment to address chronic toxicity in fish under exposure conditions being *worst-case* as compared to BYI 02960's environmental exposure estimation, namely a duration of the test that exceeds the exposure duration at field edge and a test that addresses likely most sensitive chronic endpoints in fish such as hatching and growth of juvenile stages. Thus, in presence of the ELS test the 28 day uvenile growth test was not considered necessary.

### Chronic toxicity of metabolites to fish \( \& \)

The toxicity of BYI 02960 metabolites was profiled in different standard species representing different taxonomic groups. The ecotoxicity pattern obtained and the structural characteristics of the metabolites raised no concern for toxicity against fish hence, testing was limited in order to comply with anomim to limit vertebrate testing whenever possible. In the two cases where metabolites have been subjected to an acute toxicity test in rainbow troop (DFA and BYI 02960-suconnamide) there was no adverse effect seen at the maximum tested dosesteing significantly above the expectable exposure concentrations. Hence considering the generally low toxicits of the parent substance to fish and the observations made on the 2 tested metabolites. Surther Chronic fish tests were not considered justifiable.

IIA 8.2.4 Fish early life stage toxicity test

Report: KMA 8.24/01; C.V. (2011)
Title: Viarly Life Stage Toxicity of BY V02960 rechnical to the Fathead
Minpow(Pimerhales Prometas) Under Flow Through Conditions
Report No: BRAVP033
Document No: M-409339-01-1 0 0
Guidelines. OECD Guideline 210 (1992(\$
EPA-FFRA Guidetine 72 (a), 1982
1
Deviations:
Screening of water or contaminants was not performed under GLP.

# EXECUTIVE SUMMARY

The aim of the study was to determine the offects of BYI 02960 (Sample description: TOX 08508-00 (Batch ID: 2009-000239); purity 96.2%) to fathead minnow (*Pimephales promelas*).

Eggs and fry of *imephales plemelas* were exposed in a flow-through system over a period of 35 days to nominal concentrations of 0.625, 1.25 £.50, 5.00 and 10.0 mg a.i./L (corresponding to mean measured concentrations of 0.615, 1.11, 2.05, 4.41 and 8.40 mg a.i./L). In addition, a water control and a solvent control (0.1 m) L DMP/L) were tested.

Mean measured recoveries were within the range of 82 to 99% of the nominal concentrations. Hatching rates, subjectual symptoms, fry survival and growth (length and wet and dry weight) were recorded daily. Based on analytical findings the biological endpoints are reported as mean measured figures.

The 35-day exposure to BYI 02960 technical resulted in a NOEC of 4.41 mg a.i./L and a LOEC of 8.40 mg a.i./L based on fry survival.

#### MATERIAL AND METHODS

#### A. Materials

#### 1. Test material

Test item: BYI 02960 Type of test material: Substance, technical Chemical state and description: Beige powder Batch number: 2009-000239 Sample description: TOX 08508-00

TOX 08508-00
2(5H)-Furanone, 4-[[(6chloro-3-pyrid@yl)methyl](2.2-difuoroethyl)amino] CAS name:

CAS#: 951659-40-8

fluoroethylaminolturan-2(5H)-one IUPAC name: 4-[(6-chloropyridinylmethyl)

96.2% Purity:

Storage conditions: Room temperature

Water solubility: Approximatel

#### 2. Test solutions

Vehicle:

Controls:

#### 3. Test organisms

Pimephales pronctas Species: Common name: Tathead minnow Source: În-house culture

Brine shripe (Artenia salina) starting on Day 4 Feeding during test:

4 hours old eggs in the pre-blastula, Wastula and gastrula stages Developmental stage at

√eight based on controls) Static loading: Dynamic loading: day (mean biomass based on controls)

Maintenance of culture

Temperature:

Photoperiod: 16 bours light, 8 hours dark Food: Tetramin and/or brine shrimp with 2 makes and 5 females Breeding tanks: Remarks:

#### B. Study design and method

eptember 29, 2010 1. In life dates

#### 2. Design of biological test

Pimephales prongelas were exposed to BYI 02960; (purity 96.2% w/w) in a flow through system over a period of 35 days. Testwesses were dosed via a proportional diluter with a renewal rate of approximated 10 volume Ornovers per 24 hours. Nominal concentrations were 0.625, 1.25, 2.50, 5.00 and 10.0 mg a.i. @ In addition a water control and a solvent control (0.1 mL DMF/L) were tested. Each vessel (glass vessel; & L) served as one replicate containing one egg cup and filled with 7 L soft processed water (blended pring and reverse osmosis waters). 35 eggs at initiation, thinned to 20 alevin after hatching phase, were used per replicate. Thinning of surplus alevin took place at day 5 (when at least 90% of viable control eggs had hatched), the post-hatch phase started at day 5. The static biological loading was 0.14 g/L (mean wet weight based on controls). The dynamic biological loading

was 0.041 g/L/day (mean biomass based on controls). The test was conducted with 4 replicates per treatment level.

#### 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at experimental start weekly ( $\pm$  2 days) thereafter including experimental finish from all concentrations. BYI 02960 served as analytical standard. The limit of quantification (LOQ) was 0.06 mg a.i./L.<sub>a</sub>

#### 4. Observation and measurements

Biological data and physical-chemical water parameters were assessed as indicated below in the result section.

#### 5. Statistical analysis

For each parameter analyzed (mortality, hatchability, growth), the following matistical tests were conducted:

- 1) Chi-square test to test for normality and Bartlett Vtest for homogeneity of variance. All days were analyzed without transformations.
- 2) One way Analysis of Variance (ANOVA) was used to determine if there was a significant difference between the treatment groups and the control.

If the results of the ANOVA showed significant differences (\$\old{O}\$= 0.05) then the Barterroni t-test and the William's test (if appropriate) were conducted to identify which treatment group(s) were significantly different from the control groups. The results were used to determine the No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC).

#### RESULTS AND DISCUSSION

### A. Physical and Chemical Parameters.

Measurements of physical and chemical parameters of the test solutions are summarized as follows:

24.6 to 25.0 @ (mean 24.8 @ Test temperature: pH: Dissolved xygen (mg/L) to 8.1 (mean (7.4) Dissolved oxygen (% saferation). to 98 (mean, 90) 16 hours light, 8 hours dark Photoperiod: Cool white fluorescents Light source Light/dark transition period Light intensity: 46 to 54 mg/L as CaCO<sub>3</sub> (mean: 49 mg/L as CaCO<sub>3</sub>) Hardness: 056 48 mg/L as/CaCO<sub>3</sub> (mean: 43 mg/L as CaCO<sub>3</sub>) Alkalinity: Conductivity: 163 µmfros/cm (mean 158 µmhos/cm)

### B. Analytical Findings

Analytical verification of test colutions revealed measured concentrations of 0.619, 1.11, 2.05, 4.41 and 8.41 mg a.i./1.82% to 99% of nominal) calculated as arithmetic mean. Biological results are reported as mean measured Detailed analytical results are presented in the following table:

Table:	Nominal and mean measured concentrations of BYI 02960
i abic.	110 minar and mean measured concentrations of D 11 02/00

Nominal		Measured	Concent	ration in n	ng a.i./L 1)		Mean	SD	% of	
concentration	day 0	day 8	day	day	day 28	day 35	[mg a.i./L]		nomina	ô
[mg a.i./L]			14 + 15	21 + 22						Ŋ
Control	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06	n.a	n.a.	Onja.	
Solvent	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06	Ą.	n.a.	n.a.Ç	
control		10.00	.0.00			10.00	-		11.4.	
0.625	0.72	0.60	0.59	0.52	0.72	0.70	<i>△</i> 0.62	0.0	<b>199</b>	)
1.25	1.22	1.18	0.98	0.95	1,26	1.35	<b>~</b> 1.11	0/16	×88 ×	
2.50	2.30	2.20	1.84	1.81	231	2.27	* 2.05 (	0.24	× 82,0°	"W
5.00	4.94	4.62	4.07	3.93	5.00	4.78	4.41 $\sqrt{Q}$	0.48		Š¥
10.00	9.03	9.02	7.66	7.79	§ 9.21	9:JJ	8.410	0.78	<u>34</u>	
1) Values given are arithmetic mean of two replicates per day										
n.a.= not applicab	ole	LC	Q = < 0.06	omga⊘n/L	SD = St	andard de	viation 🔻 🦯	)	ð <u>"</u> Q"	
1) Values given are arithmetic mean of two replicates per day  n.a.= not applicable  LOQ = < 0.06 mg ap /L SD = Standard deviation										
C. Biological Fi	inaings		4				) · · · · · · · · · · · · · · · · · · ·	ÕY		
Biological parar	neters we	re observe	ed as liste	d below.	. Y »	4			, O	
Biological parameters were observed as listed below.										
Biological parameters were observed as listed below.  Table: Effect of BYI 02960 on early life stage of Fimephales promelas.  Mean measured Mean percent hatch % Alexin Mean length Mean dry										
Mean measure	d Mea	n percent	hatch /	% Alevii	n 🔊 %	Fry A	Mean length	M	<b>@</b> n dry	
concentration		¢.	9	survival	S, L		\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\		ght [mg]	
[mg a.i./L]	day	4 0,0	lay 5	aay 5 A		ay 35√	day 35		day 35	
Control	43.	6	80.78	≈ 80.0 <sup>4</sup> ©		938	21.9	d <sup>y</sup>	31.6	

<sup>1)</sup> Values given are arithmetic mean of two replicates per day

### C. Biological Findings

Mean measured concentration	Mean per	. O' .	% Alevin survival	% Fry survival	Mean ength	Mean dry weight [mg]
[mg a.i./L]	day 4	aday 5	aay 5 🗬	aay 35	day 35	day 35
Control	43.6	Ş 80.7∀	80.0 <sup>0</sup>	93.8	a 21.9 C	31.6
solvent control	52.1	<b>85</b> ,0	O 8249 7	y 9 <b>3</b> 4.8 🦠	<b>\$ 2</b> 2.4	33.1
0.619	20.0	\$0.0 £	<b>48.6</b>	86.3 +	~~~ <sup>7</sup> 22.5~~	34.2
1.11	27.Ĭ <sup>®</sup>	<b>△</b> 83.6	₹83.6 Ø	95,0	√ 222×	33.8
2.05	65.0	\$ 85.7	83,6	× 97,3	, 22,6	34.8
4.41	Ø21.4	<b>20</b> 7.7	D 869.0	88.8 O	<b>2</b> 2.0	33.7
8.40	38.6	×3.6 ×	82.9	87.5 *C	@ 22.5	36.1

<sup>\*</sup> statistically significant effect P = 0.03 as compared to pooled controls (Williams)

The Bonterroni t-test slowed estatistically significant difference of the lowest test concentration (0.619 mg a.i./L) in comparison to sooled control. However, the difference observed between the control and the lowest treatment group is not considered to be biologically significant because of the lack of a doseresponse relationship.

#### D. Validity Criteria

The overall survival of feetilized eggs in the controls was greater than the species-specific limits given in the guidelines. The oxygen saturation was above 60%. The water temperature did not differ by more than £ 5°C between characters of successive days. Concentrations of test item were within ± 20% of nominal.

## E. Biological Pndpoints Derived

From the results presented above the following biological endpoints can be derived:

<sup>+</sup> statistically significant effect (P = 0.05) as Ompared to pooled controls (Bonteroni), not considered to be biologically significant

NOEC (overall):	4.41 mg a.i./L
LOEC (overall):	8.40 mg a.i./L
NOEC (alevin survival day 5):	8.40 mg a.i./L
NOEC (fry survival day 35):	4.41 mg a.i./L
NOEC (percent hatch):	8.40 mg a.i./L
NOEC (time to hatch):	8.40 mg a.i./L
NOEC (growth in terms of length):	8.40 mg a.i./L
NOEC (growth in terms of weight):	8.40 mg a.i./L
NOEC (morphological and behavioural effects):	8.40 mg a.i./L

#### **CONCLUSION**

The effect of (BYI 02960) on early life stages of father minnow (*Pinephales promelas*) can be quantified as a no observed effect concentration of 441 mg a.i./L (based on fry varivity) day 35)...

### IIA 8.2.5 Fish life cycle test

Acute toxicity findings and the observed low long-term toxicity in the ELS test (8.2.4.) for B&F 02960 suggest no serious effects are expected from long term exposure of fish. BYL 02960 is only moderately persistence in water and the low potential for bioconcentration (log  $P_{ow} < 3.0$  combined with a high water solubility of ca. 3.2 g/L) suggest long term effects based upon accumulation in the fish are not anticipated. Therefore, a full-life-cycle (exposure) test would not be expected to provide additional data relevant for risk assessment in addition to that already established by the ELS test.

### IIA 8.2.6 Bioconcentration potential in fish

## IIA 8.2.6.1 Bioconcentration potential of the active substance in fish

BYI 02960 has a log ow of 1.2, therefore no study is required seconding to all major worldwide regulatory requirements.

# IIA 8.2.62 Bioconcentration potential of the metabolites, degr. & react. products

The log P<sub>ow</sub> of the metabolites of B×4 02960 are all well below 3 and therefore there is no concern for bioconcentration for the metabolites.

BYI 02960 Metabolite	Maximum Log 🗞	Reference
BYI 02960-succinamide	0.6 at p 1 5	KIIA 7.13/06
BYI 02960-a2abicyclosuccinamide 3A-salt	₹-1,3 <b>@</b> pH,5©	KIIA 7.13/07
DFA Q Q Q	- 2 at pH5	KIIA 7.13/08
6-CNA	1.98 at p 1.98	KIIA 7.13/09
	W - Y	

# IIA 8.2.7 Aquatic bioavailability/biomagnification / depuration

Although BY 02960 can be expected to be bioavailable, based upon its water solubility, it will not concentrate in bioa. It should equilibrate rapidly in the exposed organisms and depuration should also be rapid as observed in the mammalian metabolism studies. As such, the risk for biomagnification is considered raggligible.

#### IIA 8.3 Toxicity to aquatic species other than fish, aquatic field tests

Parent compound

BYI 02960 belongs to the chemical class of butenolides. It controls sucking insects with a good selectivity thus avoiding adverse effects on many other non-target organisms. From the pesticidal activity against insects it can be expected that aquatic invertebrates are potentially sensitive organisms in the aquatic environment. As such, all established tests for indicator species were performed to profile BYI 02960's toxicity to invertebrates. Crustaceans are represented by *Daphnia pugna* and the calt water species *Americamysis bahia*, a species for which the US-EPA requires testing. The midge *Chironomus riparius* was tested representing aquatic insects, a likely sensitive group within invertebrates. For these species acute and chronic tests are available significantly reducing the uncertainty for potential chronic and population relevant effects. Although it is not a curopean requirement, the shell deposition test on the marine over *Crassostria virginica* completes the darbase of BYI 02960's toxicity to aquatic invertebrates.

# Metabolites

As previously discussed (see Section 8.2) the testing strategy for the metabolites considered the relationship to the parent structure in determining the testing strategy.

For the aquatic photo-degradates testing was focused on the species which were most sensurive to the parent, while for the soil metabolites DFA and 6-CNA which are less closely related to the parent structure a wider spectrum of testing was performed.

# IIA 8.3.1 Acute toxicito to aquatic invertebrates

# IIA 8.3.1.1 Acute toxicity (24 and 48 hour) for Daphnia preferably (Daphnia magna)

Report:	KIJA 8.3.10/01; C.S. C.V.Q.2009)
Title:	Acute Toxicity of BYI 02960 to Dophnia magna Under Static Conditions
Report No:	KBRVP032 V V V
Document No:	M-357476-010 V V V
	OECD Guideline 202
	EPA ORP 72-2
Deviations:	None
GLP:	Yes (certified laboratory)
GLP:	Routing creening of water for contaminants was not performed under GLP.

# EXECUTIVE SUMMARS.

The aim of the study was to determine the acute effects of BYI 02960 (Sample description: TOX 08508-00 (Batch 1D: 2009-000239); parity 96.2%) to Daphnia magna.

Daphina magna ((6 replicates of 5) <24 hour of neonates) were exposed in a static system over a period of 48 hours to nominal soncentrations of 80 mg a.i./L (corresponding to analytically verified concentration of 77.6 mg a.f.L). In addition, a water control and a solvent control were tested. There were six replicates of five daphna each in the control, solvent control and the treatment. This study was run as a limit test at a single treatment concentration at the limit of solubility in the test system, which was determined to be approximately 80 mg a.i./L.

The mean measured recovery of the single treatment level was 97% of the nominal concentration. Results are based on the mean measured test concentration.

Immobility was defined as the inability to swim within 15 seconds after gentle agitation of the test vessel even if the organisms can still move their antennae. Sublethal and behavioral effects were also assessed during the course of the study.

Following 48 hours of exposure neither immobilisation nor sublethal effects were observed at any lest level. Therefore, the 48-hour-FCcc was determined to 1 and 27 feet. level. Therefore, the 48-hour-EC<sub>50</sub> was determined to be > 77.6 mg a.i./L, the 48-hour-NOEC was 77.6 mg a.i./L.

# MATERIAL AND METHODS

### A. Materials

# 1. Test material

BYI 02960 Test item: Type of test material: Substance, technic Beige powder Chemical state and description: 2009-000239 Batch number: TOX 08508-00 Sample description

CAS name: 2(5H)-Furamone,

difluoro thyl)amino]

CAS#: 951659-40-8

IUPAC name: 4-[46-čhloropyridin-3

Purity:

Solubility:

Storage conditions:

# 2. Test solutions

Vehicle:

Concentration of vehic Controls: Evidence of undisso

# 3. Test organisms

Species: Common name: Water flea√

BS072908 (Subeultuse S-1212 Strain:

Source: Inhouse cultur since PUL 2008; origin from

Age at study initation;

Feeding during test: Maintenange of culture:

Temperature: Photoperiod:

Food. udoffrchneciella subcapitata and/or blended fish flake food

# B. Study design and method

May 12 0 14, 2009 1. In life da

# 2. Design of biological test

Daphn a magna (<24 Mour old neonates) were exposed to BYI 02960 (purity 96.2% w/w) in a static system over a period of 48 hours. The nominal concentration of the limit test was 80 mg a.i./L.

In addition, a water control and a solvent control (0.1 mL DMF/L) were tested. Each vessel (glass beaker; 250 mL) served as one replicate filled with 200 mL hard water (blended spring and reverse

osmosis). Biological loading rate was 40 mL/animal. The test was conducted with 6 replicates per treatment level, consisting of 5 daphnids per replicate.

# 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at 0 hours and 48 hours from all concentrations. The limit of quantification (LOQ) was 0.62 mg a.i./L.

## 4. Observation and measurements

Immobilisation of daphnids, intoxication symptoms and physical-chemical water parameters were assessed as indicated below in the result section.

# 5. Statistical analysis

No statistical calculations were necessary to determine the EC50 for this study. The NOEC and LOFF were empirically determined based upon observation data including lethal and subletial effects.

# RESULTS AND DISCUSSION

# A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test solutions are summarized as follows

Test temperature:	Mean: 19.9°C (frange: 19.6 to 20.2 °C) 5
pH:	
Dissolved oxygen (mg/L):	*8.1 <b>(8</b> 8.3 <b>(9 (9 (9 (9 (9 (9 (9 (9</b>
Dissolved oxygen (% saturation at	89 to 93
20°C): ৺	
Aeration used:	No S O O O
Photoperiod:	16 Mours light, 8 hours dark \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \
Light source	Cool white fluorescents O
Light/dark transition period:	Significant Signif
Light intensity:	540 to 600 ltd 5
Hardness:	Mean: 169 mg/L (Tange: 164 to 178 mg/L)
Alkalinity:	Mean: 136 mg/L/range: 129 to 50 mg/L)
Conductivity: S	Mean 382 μm ros/cm Grange 359 to 426 μmhos/cm)

# B. Analytical Findings

Analytical verification of test solutions revealed a mean measured concentration of 77.6 mg a.i./L (97% of nominal) calculated as arithmetic mean. Biological results are reported as mean measured. Detailed analytical results are presented in the following table:

Table: Nominal and measured concentrations of BYI 02960

Ī	Nominal	Day Day	New)	y Day 2	(Old)	Mean	Mean
	Concentration	Measured∀	Percent		Percent	Measured	Percent of
	(mag a.i./L)	∕ (mg a,i-⁄L)	Nominal	(mg a.i./L)	Nominal	(mg a.i./L)	Nominal
ſ	≫ 80	87	× 102%	74	92%	77.6	97%

# C. Biological Dindings

Observations on immobilisation and sublethal intoxication symptoms are listed as follows:

No subjetual behavioural changes were observed.

Table: Effect of BYI 02960 on immobilisation of *Daphnia magna* 

	No of	Observation period					0
Treatment	No. of	4 ho	ours	24 h	ours	48 h	ours $\mathscr Q$
[mg/L]	organis ms	# immob.	% mortality	# immob.	% mortality	# immob.	mortality
Control	30	0	0.00	0	0.00	<b>~</b> 0	L 0.00
Solvent control	30	0	0.00	0	0.00		0.00
77.6	30	0	0.00	0	0.00,	0 . Ô	* <b>6</b> 00 /

# D. Validity Criteria

The validity criterion of control mortality less than 10% is fulfilled. The validity coterion of oxygen saturation above 60% is fulfilled.

# E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived

48-hour-figures:

EC50:

Highest concentration with no effect (NOE)

Lowest Concentration With an Effect (LOEC)

· 77.6 mg a.t./L

77.6 mg a,i. L

≥ 7.6 mg/a.i./

# **CONCLUSION**

The acute effect of BYI 02960 on *Daphnia Magna* can be quantified as a 48-hour-EC<sub>50</sub> of > 77.6 mg a.i./L (limit test). The highest concentration with no observed impobilisation and no sublethal behavioural effects can be set to 77.6 mg a LL the limit test concentration.

Report: \$\sqrt{\kappa \text{IIA 8.3.1.1/02;}}\$
Title: Acute toxicity of BC & AB60481 to the water Rea Daphnia magna in a static laboratory
test System - LIMIT TEST & O O
Report No: 6 EBRVP0 4 6
Document No:   M-409226-01-20
Guideline 202 O S S
EC Council Regulation No 440/2008, Method C.2 (2008)
Deviations: None V V V V
GLP: Yes (certified beboratory)
GLP: Ves (certified beboratory) Screening of dilution water for contaminants was not performed under GLP.

### EXECUTÆVE SUMMÆRY

The aim of the study was to determine the acute effects of sodium difluoroacetate (Na-salt of BYI 02960 DFA) (Sample description TOX 08988 O); code: BCS-AB60481; purity > 99.0% w/w) on mobility of *Daplaia magna* over 48 bours under static exposure conditions. The study was performed as a limit test of a single concentration of 10 mg pure metabolite/L

Daphnia magna (24 hour old no nates, 10 replicates of 5 individuals)) were exposed in a static system over a period of 38 hours to no minal concentrations of 10 mg/L (corresponding to analytically verified concentrations).

Immobilisation and sublethal behavioural effects were recorded as endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. Due to the absence of treatment

related effects up to a nominal concentration of 10 mg/L, the EC<sub>50</sub> for immobilisation after 24 and 48 hours of static exposure was higher than 10 mg BCS-AB60481/L.

# MATERIAL AND METHODS

# A. Materials

### 1. Test material

Test item: Sodium difluoroacetate (Na-salt of BYI 02960-DFA,

Substance, technical (pure metabolite) Type of test material:

Chemical state and description: White powder Batch number: BCS-AB60841-01

BCS-AB60481 Code: Sample description: TOX 08988-01 > 99.0% w/wPurity: Storage conditions: +10 to +309

# 2. Test solutions

Vehicle:

Ulfrasonicated for Method of preparation:

magnetic stirrer

Controls: Water Control Evidence of undissolved material:

# 3. Test organisms

Species:

Common name:

Bradley, University of Shelfield Strain:

Source:

Age at study initiation

Feeding during test

Maintenance of culture Temperature:

Photoperiod:

Food: of the green alga Desmodesmus subspicatus

# B. Study design and method

# 10 to February 15, 2011

2. Design of biological test Daphnia morgna (<24 hour old peonates) were exposed to sodium difluoroacetate (Na-salt of difluoroacetic acid, DEA) (code: BCS-AB69481; parity > 99.0 % w/w) in a static system over a period of 48 hours. Nominal concentrations were 10 mg/p.m./L. In addition a water control was tested. Each replicate vessel was a glass beaker (100 mL) filled with 50 mL Elendt M7. Five daphnids were used per replicate. Biological loading rate was 10 mL/animal. The test was conducted with 10 replicates per treatment level.

# 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at 0 and 48 hours from all concentrations. HPLC-MS/MS was used as analytical method. The limit of quantification (LOQ) was  $0.998 \mu g/L$ . The range of linearity was  $0.11 \mu g/L$  to  $11 \mu g/L$ .

## 4. Observation and measurements

Immobilisation of daphnids, intoxication symptoms and physical-chemical water parameters were

Nominal ~		(New)	Day 2	(Old)
Concentration	O' Measured 2		Measured	
[mg p.m./L]	kong p.mk/L /	% Nominal	[mug p.m./L]	% of Nominal
Control	0.000998	NA V	0.000998	NA
* 10	\$\frac{1}{2}\text{\$\tilde{0}.1}\$ \$\tilde{\chi}\$	\$ \$\frac{1}{2}\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\exitt{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\exitt{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\exitt{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\exitt{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\exitt{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\exitt{\$\text{\$\text{\$\text{\$\text{\$\text{\$\exitt{\$\text{\$\exitt{\$\exitt{\$\text{\$\text{\$\text{\$\exitt{\$\text{\$\text{\$\text{\$\tex{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$	10.3	103

# C. Biological Findings

Observations of immobilization and subletral intoxication symptoms are listed as follows:

Observed immobilization Table:

Nominal Test 🧳	Exposed S	) Immobilise	d Daphnids
Concentration &	Daphuids Daphuids	24 h	48 h
mg p.m./L]		[n]	[n]
Control	50	0	0
100	5000	0	0

No sublethal behavioural changes were observed.

The validity criterion of control mortality less than 10% is fulfilled. The validity criterion of oxygen saturation above 60% is fulfilled.

# E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

EC<sub>50</sub> (48 h)

> 10 mg p.m./L

### **CONCLUSION**

The acute effect of sodium difluoroacetate (Na-salt of difluoroacetic acid) (BCS-AB60481) on *Dophnia magna* can be quantified as  $EC_{50} > 10$  mg p.m./L. Observations on sublethal effects revealed no abnormal behaviour of the exposed daphnids over the entire exposure period of 48 hours.

The following study was performed for the registration of acetamiprid and is the property of Nippon Soda Co. Ltd, access to the study has been granted. The study has been evaluated during the Annex inclusion of acetamiprid, therefore only a very front summary of the study conclusion is repeated here. Note- the test compound IC-0 is identical to 6-CNA.

Report:	KIIA 8.3.1.1/03
Title:	IC-0 - Acute toxicity (48) hours) to daplinds (Duphnia Tagna) under semi-state conditions
Report No:	C007748 (Study report number: SAS97045)
Document No:	M-196569-01-1
Guidelines:	OECD Guidefine No. 202 (1984)  EEC Directive 92/69 - Method C 2/(1992)  EPA / FIFRA Guideline 2-2 (1985)
<b>Deviations:</b>	None V V V V V
GLP:	Yes (certified paboratory)  Routine screening of water for contaminants was not performed under GLP.

### EXECUTIVE SUMMARY

The aim of the study was to determine the acute effects of 6-chloronicotinic acid (6CNA; Nisso Code IC-0; purity 99.7%) to Paphria magna.

Daphnia magna (<2 hour old negrates) were exposed on a semi-static system over a period of 48 hours to nominal concentrations of 6.5 12.5 25.0, \$0.0, and 100.0 mg/L (corresponding to analytically verified concentrations of 6.0 11.9; \$3.9, 47.2 and \$5.1 mg/L; 94.4 to 95.6 % of nominal). In addition a water control was tested.

Immobilitation was used to determine the empoints Based on analytical findings the biological endpoints are reported as mean measured figures. The 48-hour-EC50 was > 95.1 mg/L, the 48-hour-NOEC was determined to be 95.0 mg/L.

# IIA 8.3.1.2 Acute toxicity 24/48 h) for representative species of aquatic insects

As the toxicity of BYI 02960 to Daphnia magna is low obtaining additional data on a second species within the group of aquatic invertebrates was considered advisable (see also Guidance Document on Aquatic Ecotoxicology, 2002). As aquatic insects were shown to be sensitive to parent molecule, studies were also performed with the metabolites.

The acute tests were performed before the test guideline OECD 235 for the acute immobilization was finalized. As such, some minor deviations from the test guideline may be noticeable However, any minor deviations are considered unlikely to have influenced the outcome of the study.

Report:	KIIA 8.3.1.2/01; (2011)	
Title:	Acute toxicity of BYI 02960 (tech.) to larvae of	of <i>Chironomus riparius</i> in a 48 h statie
	laboratory test system	
Report No:	EBRVP026	
Document No:	M-414739-01-2	
<b>Guidelines:</b>	No specified guideline; study is performed a	according to general aspects as quoted
	under OECD Guideline No. 2002	
<b>Deviations:</b>	According to test system	
GLP:	Yes (certified laboratory)	
	Screening of water for contaminants was no	ot performed under GLP as described
	in the study report	

## **EXECUTIVE SUMMARY**

The aim of the study was to determine the acute effects of BV1 02960 (Sample description: TOX 08508-00 (Batch ID: 2009-000939); purity 96/2%) on larvay of Chronomus rigarius, &

Chironomus riparius (first instars, less than 2 to days old, 40 per test concentration) were exposed in a static system over a period of 48 hours to nominal concentrations of 3.125, 6.25, 12.5, 23.0, 50.0 and 100 µg a.i./L. In addition a water control was tested. Four replicates, containing 10 animals each, were tested for each test item concentration and the control.

The analytical findings in all freshly prepared test levels on day 00n reference to nominal concentrations ranged between 97.0 and 103 % (average 101 %). In aged test levels on day 2 analytical findings ranged between 101 and 107 % (average 104 %) of nominal. Due to the high recoveries at the beginning of the exposure and the analytical findings after 2 days all results are based on nominal concentrations.

The concentration causing 50% mmolestry to larvae of Chironomus riparius (48h -EC50) was determined and occurrence of symptoms was recorded and evaluated after 48 hours of exposure.

Following 48 h of exposure 0, 0, 25, 25, 30, and 85% immobilisation was observed among daphnids exposed to the 3,125, 625, 1255, 25, 50.0 and 100 µg a Lt treatment levels, respectively. No immobilisation occurred in the water control.

Therefore, the 48-hour-EC50 was 61.7 bg a.i.4 (95% confidence limits 41.4 to 109  $\mu g$  a.i./L).

# MATERIAL AND MESTHODS

# A. Materials

1. Test material

Test item: BYP02960

Type of test material:

Chemical state and description:

Batch number 2009-000239

Sample description:

Content 96.2% w/w

Storage conditions:

Substance, technical Beige powder

2009-000239

TOX 08508-01

96.2% w/w

25  $\pm$  5°C

## 2. Test solutions

Vehicle: None

Controls: Water control

Evidence of undissolved material: None

# 3. Test organisms

Species: Chironomus riparius Common name: 1st instar midge larvae Strain: From University of Inhouse culture since 2006 Source: Age at study initiation: Less than 2 to 3 days old

0.01 mL of an agreeous fish food si Feeding during test:

added at test start

Maintenance of culture:

Temperature:

Photoperiod:

The hatched harvae are fed with green algae and an aqueous suspension of a plant material based fish food (Tetra Phyllod) Food:

# B. Study design and methods

Januar 14 to June 08, 2011 1. In-life dates:

# 2. Design of biological test

Chironomus riparius (first instar less than 2 to 3 day cold) were exposed to BYI \$2960 (purity 96.2% w/w) in a static system over a period of 48 hours. Nominal concentrations were 3.125, 6.25, 12.5, 25.0, 50.0 and 100 μg a.i. (Figure 10) in addition, a water control was tested. Fach vessel (glass beakers) served as one replicate filled with 25 mL Elendt-medium (MJ). Ter darvae were used per replicate. The test was conducted with 4 replicates per treatment level.

# 2. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at day 0 and day 2. HPLC-MS/MS was used as analytical method. The limit of quantification (LOQ) was 0.5035 mg/L. The range of linearity was 0.05 pc/L to 1/4 µg/L

# 3. Observation and measurements

Immobilisation of midge lawae, intoxication symptoms and physical-chemical water parameters were assessed a condicated below in the result section

The EC<sub>x</sub> values were determined with Probit analysis using linear maximum likelihood regression (Chi<sup>2</sup>-Test). NOFC and LOEG were calculated with Fisher's Exact Binomial Test with Bonferroni

# A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test solutions are summarized as follows:

Test temperature:  $20.6^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$  pH: 7.9 to 8.0 Dissolved oxygen (mg/L): 8.2 to 8.4 Dissolved oxygen (% saturation): 90 to 94

Light/dark transition period: 16 h light / 8 h dark Light intensity: 500-1000 lux

# **B.** Analytical Findings

Analytical verification of test solutions revealed that concentrations in all freshly prepared test levels of day 0 ranged between 97.0 and 103 % of nominal encentrations (average 101 %). In aged test levels on day 2 concentrations ranged between 101 and 107 % (average 104%) of nominal Biological results are reported as nominal. Detailed analytical results are presented in the following table:

Table: Nominal and measured concentrations of By 02960

Nominal concentrations	Dà	y/0 / V . V		y 2 V
μg a.i./L	μg a.⊋L 《ຶ່	%of nominal	μ <b>g@.i./</b> L	of nominal
Control	<@\$035 @*	~ - ~ ~	<b>30</b> .503 <b>5</b>	
3.125	₹3.03 ₺	\$ <b>39</b> 7 8	3.15	) <b>™</b> 101
6.25	@ 6.0 <sup>2</sup>	97 , © "	6.PT &	<b>%</b> 103
12.5	12.9	103	.©	O 106
25.0	25.2	7 1000°	26.7	<b>5</b> 107
50.0	51.6 👸 🛴	© 403 °	₩ 5 <u>2</u> .\$*	104
100	103	©103 O	( 104 6	104
Ğ	Wean: 101%	ofmominal? (	Mean; 104%	6 of nominal

# C. Biological Findings

Observations of immedilisation and Sublethal interication symptoms are listed as follows:

Table: Effects of BYI 02960 on Chironomus riparius at day 2

		7		4	
nominal concentr (μg a.i./L)	ra@ons (5)	introduced %	immobile	% mortality	symptoms observed
Control	,1		0 0	0.0	no
3.125		× 20°40 ×		0.0	no
, <b>62</b> 5		400	O' 'Ø'	0.0	no
12.5		~ 40° Q	<b>6</b> 1	2.5	no
25.0		<b>7 40 9</b>	<b>4</b> 1	2.5	no
50.0	~~~ Q	40	12	30*	no
100	\$' A	40,7 %	34	85*	yes**

<sup>\*</sup> significantly different from the control (α < 0.05)

The sensitivity of the test organisms was rested with 3,5-dichlorophenol as a toxic reference on a regular basis.

# D. Validity Croteria

All wandity enteria were met. The validity criterion of control mortality less than 10% is fulfilled. The validity enterior of oxygen saturation above 60% is fulfilled.

<sup>\*\*</sup> reduced mobility

# E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

Biological endpoints

reported as: Nominal concentration

48-hour-figures:

61.7 μg a.i./L (95% confidence limits 4.4 to 109 μg a.y. EC<sub>50</sub>:

NOEC: 25 μg a.i./L#

30.0 μg a.i./L (95% confidence limits 7.9 to 44.0 β a.i./L EC<sub>10</sub>:

# **CONCLUSION**

The acute effect of BYI 02960 on Chironomus siparius can be quantified as a 48-hour-EC 61.7 μg a.i./L (95% confidence limits 41.4 to 109 μg/a.i./L) The highest concentration with ng observed immobilisation and no sublethal terravioural effects can be sen to 25 and

Report:	KIIA 8.3.1,2702; (2011) 7 2 5 5 5
Title:	Acute toxically of BYI 02%0-successamide of larvacof Chisonomics riparities in a 48 h
	Acute toxicity of BYI 02960-successamide to larvae of Chironomic riparius in a 48 h static laboratory test system
Report No:	
Document No:	M-417386-64-2 & & & & & & & & & & & & & & & & & & &
Guidelines:	No specified guideline; andy is performed according to general aspects as quoted
	under OECD Guidelige No. 202
Deviations:	According to test system
GLP:	Yes (certified laboratory)
GLP:	Yes (certified laboratory) Servening of water for contaminants was not performed under GLP as described
ق ،	in the study report

The aim of the study was to determine the acute effects of BYI 02000 – succinamide (Sample description: TOX 09343-00 (Batch 1D: BCOO 6329-2-10); purity 97.8% w/w) to larvae of Chironomus riparius.

Chironomus riparius (first instars, less than 2 to 3 days old 40 per treatment level) were exposed in a static system over a period of 48 hours to nominal concentrations of 26, 36, 51, 71 and 100 mg pure metabolite (p.m.)/L, respectively. In addition, a water control was tested. Four replicates, containing 10 animals each, were tested for the test item concentrations and the control.

The analytical findings in all freshly prepared test levels on day 0 in reference to nominal concentrations ranged between 103 and 106 % (average 104%). In aged test levels on day 2 there were analytical findings between 102 and 107% (average 104%) of nominal. Due to the high recoveries at the beginning of the exposure and the analytical findings after 2 days, all results are based on nominal concentrations.

As the primary endpoint, a concentration causing 50% immobility to larvae of *Chironomus riparius* (48 ) EC<sub>50</sub> was determined. Additionally, possible occurrence of symptoms was recorded and evaluated after 48 hours of exposure.

Following 48 h of exposure 0, 0, 2.5, 2.5, and 15% immobilisation was observed among daphnids exposed to the 26, 36, 51, 71 and 100 mg p.m./L nominal treatment levels, respectively. No

<sup>38.4</sup>  $\mu g$  a.i./L (95% confidence limits 15.3 to 54.5  $\mu g$  a.i./L) # based on not statistically significant immobilization at the 50 and 100  $\mu g$ /L test concentration and absence of statistically significant effects at 12.5 and 25  $\mu g$ /L..

immobilisation occurred in the water control groups. There were no other abnormal signs indicative for toxicity observed during the test period of 48 hours in the control or the treatments.

The 48-hour-EC $_{50}$  was  $\geq$  100 mg p.m./L and the 48-hour-NOEC was determined to be 71 mg p.m./L based on the statistically significant findings at 100 mg/L.

# **MATERIAL AND METHODS**

## A. Materials

# 1. Test material

BYI 02960 - succinamede (BCS-CR Test item: Type of test material: Substance, technical (pure metabo

Chemical state and description: White powder Batch No .: BCOO 6329 Sample description: TOX 09343 Purity:

Storage conditions:

# 2. Test solutions

Vehicle: Controls: Evidence of undissolved material:

# 3. Test organisms

Chi**zo**ĥomus ripariyl Species: 1 nstar Oridge larvae Common name: Strain: From University of Inhouse cultinge since 2006

Age at study infration Less than 2 to 3 days old

Feeding during test:

Maintenance of cultur

Temperature: hour fight, Shour dark Photoperiod:

The Catched arvae are fed with green algae and an aqueous suspension of a Food: based fish food (Tetra Phyll®).

# B. Study design and methods

1. In kite dates: ıgust

## 2. Design of biological test

Chironomus riparius (Tess than 2 to Vays pld) were exposed to BYI 02960 – succinamide (purity 97.8% w/w/in a staric system over a period of 48 hours. Nominal concentrations were 26, 36, 51, 71 and 100 or pure netabolite (p.m.)/L, respectively. In addition a water control was tested. Each vessel (glass keakers served as one replicate filled with 25 mL Elendt-medium (M7). 10 larvae were used per replicate. The test was conducted with 4 replicates per treatment level.

# 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at day 0 and day 2 from all concentrations. HPLC-UV was used as analytical method. The limit of quantification (LOQ) was <0.273 mg/L. The range of linearity was 0.011 to 2.180 mg/L.

# 4. Observation and measurements

Immobilisation of midge larvae, intoxication symptoms and physical-chemical water parameters assessed as indicated below in the result section.

# 5. Statistical analysis

The ECx values were determined with Probit analysis using linear maximum likelihood regression. (Chi2-Test). NOEC and LOEC were calculated with William's test. Statistical Software "ToxRator Professional", version 2.10.05 was used. Professional", version 2.10.05 was used.

# RESULTS AND DISCUSSION

RESULTS AND DISCUSSION			
A. Physical and Chemical Parameters			arized as follows:
Measurements of physical and chemical pa	rameters of the fest	solutions are summ	arized as follows:
Test temperature: 20.2 pH: 7.2 to	© to 20,7 °C		
Dissolved oxygen (mg/L):			
Dissolved oxygen (% saturation): \$\alpha\$86 to	88 0 0 0		
	ight /8 h dags		)
Light intensity.	667 Lux		9
Hardness:	grig/L CoCO3 "O"		, Q
Alkalinity: $\sim 33.4\%$	ng/L <b>G</b> aCO3 4		
<i>←</i> 1			Z Z
B. Analytical Findings		0'	
mary tical verification of test solutions on	day 0/revealed meas	sured concern ation	s between 103 and
106 % (average 004 %) of nominal. In age	d fest levels on day?	2 there were analyti	cal findings between

106 % (average 004 %) of nominal. In aged 10st levels on day 2 there were analytical findings between 102 and 107% (average 104%) of nominal. Biological results are reported as nominal. Detailed analytical results are presented in the following table:

Table: Nominal and measured concentrations of BYI 02960 - succinamide

Nominal concentrations	Da Da	y 0, % O	D.	Day 2
mg p.m./比/	mg p.m./L		mg p.m./L	% of nominal
control O	© <0.293		© <0.273	
26	2 (2 kg/X)	Ø 03 D	27.7	107
△36	\$7.4	\$\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	37	103
51	Q 52.7	2 103 <sub>7</sub>	52	102
71	73.8	y <sub>1</sub> 04	75	106
100	, × 05 Q	<b>3106</b>	104	104
, , , , , ,	Mean: 1,04%	6 of nominal	Mean: 104	1% of nominal
		<b>Q</b> *		



# C. Biological Findings

Observations on immobilisation and sublethal intoxication symptoms are listed as follows:

Table: Effects of BYI 02960 – succinamide on *Chironomus riparius* at day 2

nominal concentrations (mg p.m./L)	introduced	immobile	% mortality	symptoms observed
Control	40	0	0.0	Ano A
26	40	0	0.0	, O no 🔊 🔍
36	40	0 Pg	000	no y
51	40	1 💎	<b>2</b> 2.5	
71	40	1,	2.5 ×	J Mo
100	40	4®"	15* C	no p

<sup>\*</sup> significantly different from the control ( $\alpha$ < 0.05), p.m. pure metabolite

# D. Validity Criteria

The validity criterion of control mortality less than 70% is culfilled. The validity criterion of caygen saturation above 60% is fulfilled.

# E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

Biological endpoint	
reported as:	A HOLIMITAL A CO. O
48-hour-figures:	
EC <sub>50</sub> :	> 100cmg p.m./L
NOEC:	71 mg p.m 🕰 🔎 🧳 🐰
EC <sub>10</sub> :	
EC <sub>20</sub> :	\$9.2 mg p.m./L \$5% confidenc@limits \$1.5 to 321.9 mg p.m./L)

### CONCLUSION

The acute effect of BYI 02960 — succinatride on Chironomus riparius can be quantified as a 48-hour-EC50 of 100 mg p.m./C. The highest concentration with no observed immobilisation and no sublethal behavioural effects (NOEC) can be set to 70 mg.p.m./L.c.

Report:	KIJA 8.3.4 2/03; (2012)
Title:	Acute toxicity of BYI 02960-azabicyclosuccinamide (BCS-CS64875) to larvae of <i>Chiffenomus riparius</i> in a 48 h static laboratory test system
Report No. >	EBRV 207
Document No:	M-424404-01-1 0 ~
Guidelines:	QECD Gridelin 235 (2011)
<b>Deviations:</b>	None & Q &
GLP:	Yes Certific laboratory)
	Septeming of water for contaminants was not performed under GET as
	@scriber in the study report

# EXECUTIVE SUMMARY

The objective of this 48 hour (h) toxicity test was to evaluate the acute immobilisation to larvae of Chironomus riparius (1st instar) caused by BYI 02960-azabicyclosuccinamide (BCS-CS64875; Sample description: TOX 09342-00 (Batch ID: SES 11732-3-1); purity 48.0 % w/w).

Midge larvae (less than 2 to 3 days old, 30 per test concentration) were exposed in a static system over a period of 48 hours to concentrations of 26, 36, 51, 71 and 100 mg pure metabolite (p.m.)/L. In addition a water control was tested. Six replicates, containing 5 animals each, were tested for the test item concentrations and the control.

The analytical findings in all freshly prepared test levels on day 0 in reference to nominal concentrations ranged between 107 and 112% (average 110%). In aged test levels on day 2 analytics findings ranged between 117 and 120% (average 119%) of nominal. Due to the high recoveries with beginning of the exposure and the analytical findings after days, all results are based on nominal concentrations.

24 and 48 hours after test initiation, the number of improbilised larvae (animals showing no swimming movements within 15 seconds after slight agitation of the vessel were recorded for each test sessel separately with a binocular. Additional observations for sub-lethal effects were performed and recorded for each test vessel separately. Significant features of the test medium (e.g. Gresence of undissolved material) were also noted.

The concentration causing 50 % immobility to larvae of Chirohomus Trarius 48 ho EC504 was determined. Additionally, possible occurrence of symptoms was recorded and evaluated after 48 hours of exposure.

After 48 hours of exposure 0, 0, 2,3, 3,3, and 20% immobilisation was observed in the 26, 36, 51, 71, and 100 mg p.m./L treatment level, respectively. No immobilisation occurred at controllevel.

The 48-hour-EC50 was >100 mg pm./L, the 48-hour-NOEC was determined to be If mg p.m./L based on the statistically analysis of the findings.

# A. Materials

# 1. Test material

Test item: %

Substance, technical (nectabolite) Type of test material:

Chemical state and desc ULight⊕rown Jiguid 🦔 Batch number: Sample description.

Storage conditions: 03-22, storage at  $5 \pm 5$ °C

### sõľutions

Content:

Vehicle:

Controls:
Evidence of und solved material.

3. Test organisms

Species

Common name:
Strand Chironomus riparius 1st instar midge larvae Strain: From University of Source: Inhouse culture since 2006 Age at study initiation: Less than 2 to 3 days old

Feeding during test: 0.01 mL of an aqueous fish food suspension (50 g Tetra Phyll/L)

added at test start

Maintenance of culture:

Temperature:  $20 \pm 2$ °C.

Photoperiod: 16-hour light, 8-hour dark

Aqueous suspension of a plant material based first food (Tetra Hyll®) Food:

# B. Study design and methods

1. In life dates: 25 November – 12 December 2011

# 2. Design of biological test

Chironomus riparius (less than 2 to 3 days old) were exposed to B @ 02960-azab cyclosuccinamide & (purity 48.0% w/w) in a static system over a period of 48 hours Nominal concentrations were 0 (control), 26, 36, 51, 71 and 100 mg pure metabolite (p/m.)/L, respectively. Each vessel (glass beaker) served as one replicate filled with 10 mL Elendt-medium (M7).

The test was conducted with 6 replicates per treatment level

# 3. Method of analytical verification

For analytical verification of the test tiem concentrations samples were taken at day 0 and day 2 HPLC-MS/MS was used as analytical method. The limit of quantification

# 4. Observation and measurements

Immobilisation of midge largae, intoxication symptoms and physical-opemical w ater arameters were assessed as indicated below in the result section

# 5. Statistical analysis:

The ECx values were determined with Probit analysis using linear maximum likelihood regression (Chi2-Test). NOEC and NOEC values were calculated with the U-test after Bonferroni-Holm and the William's t-tes Statistical Software Tox Kat Profession 2, 10.05 was used.

RESULTS AND DISCUSSION

# A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test solutions are summarized as follows:

Test temperature: pΗ: Dissolved oxygen (mg/L) Light/dark transition period

Light intensity:

# B. Analytical Findings

The analytical fundings in all freshly prepared test levels on day 0 in reference to nominal concentrations anged between 107 and 112% (average 110%). In aged test levels on day 2 analytical findings ranged between 197 and 120% (average 119%) of nominal. Biological results are reported as nominal Detailed analytical results are presented in the following table:

Table: Nominal and measured concentrations of BYI 02960-azabicyclosuccinamide

Nominal	Day 0		Day 2	
concentrations [mg p.m/L]	Analysed conc. mean of two analyses each [mg p.m./L]	% of nominal	Analysed conc. mean of two analyses each [mg p.m./L]	% of some
control	< 0.631	-	< 0.631	
26	27.8	107	30.3	_117 ×
36	40.2	112	43.1.4	120
51	57.0	ھ 112	60	7 1 18 V
71	79.0	111	<b>85</b> .0	)
100	109	109	<b>√</b> 20 <b>√</b>	₩20 ×
Average		1100		<b>₹ 119</b> 0°

# C. Biological Findings

Observations on immobilisation and sublethal Octoxication symptoms are

Effects of BYI 02960-azabicyclosuccinamide on Chironomus riparius at day

	r .			
		mbe of larta	e o y	
Treatment [mg p.m./L]	Introduced	Mobile	ammobile &	<b>%</b> mmgbility
control	30 0	30		S Q
26.0	300	<b>30</b> 6		<b>%</b> .0
36.0	30	® 30 L		0.0
51.0	V & 30 S	£ 29,		3.33
71.0	0 30 J	29	\$\tag{1} \tag{7}	3.33
100	30	<b>24</b>	6 4	20.0

The control mortality of 0.0% at 48 h will be compensated using Abbott's formula.

ich gropherol asca toxic reference on a regular basis.

# D. Validity Criteria

All validity criteria were met: Control mortality was below \$3% within 48 hours. Dissolved oxygen was > 3 mg oxygen/Lin the control and in all test concentrations

# E. Biological Endpoints Derive@

From the results presented above the following biological ordpoints can be derived:

Biological endpoints reported as. 48-hour-figures:  $EC_{50}$ : NOE/ EC<sub>10</sub>:

EC<sub>20</sub>:

8½7 mg p.m./L (95% CI 60.69 to 111.06 μg p.m./L)

EC<sub>20</sub>:

00/2.77 mg p.m./L (95% CI 83.5 to 221.27 μg p.m./L)

CONCLUSION

The acute effect of BY1 02960 azabicyclosuccinamide on *Chironomus riparius* can be quantified as a <sup>(2)</sup>7 mg p.m./L (95% CI 60.69 to 111.06 μg p.m./L) EC<sub>10</sub>:

48-hour-EC of >100 mg p.m./L. The highest concentration with no observed immobilisation and no sublethal behavioural effects (NOEC) can be set to 71 mg p.m./L based on statistical analysis.

The following study on the acute toxicity of 6-CNA to *Chironomus tentans* was reviewed during the Annex I inclusion of imidacloprid and in the DAR was concluded to be valid, a short summary of the study findings is provided here.

Report:			, C.V. (1998)	4 . 4
Title:	Acute Toxicity of 6-chlo			oprid) to
	Chironomus tentans Und	der Static Renewal C	onditions 🐥	oprid) to
Report No:	108127	Ò	S. C.	
Document No:	M-048448-01-1			
Guidelines:	American Society for T	Testing and Materia	ls (ASTM, 1987) 8	
	U.S. Environmental Pr	otection Agency (U	SEPA; 1975, 198 <b>2</b> Ç	1985) 👸 💍
<b>Deviations:</b>	None			
GLP:	Yes (certified laborator	ry)®		

### **EXECUTIVE SUMMARY**

A static limit test was conducted to determine the acute toxicity of 6-chloronicotinic acid (Batch number: 9931, lot R: 10017517, 97% a jy to Chronomus tentans.

Three replicates of ten organisms each were prepared at 1 mg/L test level, and one replicate of ten organisms was prepared for the dilution water control. Die test solutions were renewed on Day 2. Solutions of 6-chloronicotinic acid were prepared ancennal yed to determine stability after two days at room temperature. Analysis revealed recoveries of 99.2 and 99.2% of Day 0 injections and demonstrated stability of the test item in water over two days.

The primary measure for acute toxicity was nortality. Observations for Chironomas survival, sublethal and behavioral effects were recorded daily.

One larva was found dead in the control level on Day 3. No mortalities or sublethal effects were noted at the 1 mg p.m. O test concentration during the exposure period.

Therefore, the lowest observed-effect-concentration (LOEC) was greater than 1 mg p.m./L and the noobserved-effect-concentration (NOEC) was 1 mg p.m./L. The 96-hour Chironomus tentans LC50 was greater than 1 mg p.m./L.

# IIA 8.3.1.3 Acute toxicity for representative species of aquatic crustaceans

Additional data on crustacear species other than Daphnia magna representing the freshwater biocoenosis are not available. However, the saltwater sprimp Americamysis bahia is also a data requirement under US EPA guidelines and was examined using an acute flow-through test. The data are summarized under point 8.11 "marine species".

# IIA 8.3.1.4 Acute toxicity for repr. species of aquatic gastropod molluscs

In general, gastropod prolluses are considered to be significantly less sensitive than Daphnia and only in cases where directorse on parface water is intended, data for molluscs are required. BYI 02960 will not be applied to surface water; nevertheless, some evidence for the potential risk to additional invertebrates can be obtained from the available shell deposition test on the marine oyster Crassostrea virginica. This test is summarized under point 8.11. "marine species".

### **IIA 8.3.2** Chronic toxicity to aquatic invertebrates

As discussed above chronic tests on aquatic invertebrates were performed for BYI 02960 and for the ... metabolites, BYI 20960-succinamide, DFA and 6-CNA.

### **IIA 8.3.2.1** Chronic toxicity in Daphnia magna (21-day)

metabolites, BYI	20960-succinamide, DFA and 6-CNA.  Shronic toxicity in Danhnia magna (21-day)
IIA 8.3.2.1 C	Chronic toxicity in Daphnia magna (21-day)
Report:	KIIA 8.3.2.1/01; (2011)
Title:	Effects of BYI 02960 (tech.) on development and reproductive output of the waterflead Daphnia magna in a static-renewal laboratory test system
Report No:	EBRVP209
Document No:	M-414066-01-2
Guidelines:	OECD-Guideline No. 211  EC Council Regulation No. 440/2008, Method C.20  U.S. FIFRA72-4 (1982)  U.S. EPA- OPPTS Guideline \$50.1300
<b>Deviations:</b>	None S S S S S S S S S S S S S S S S S S S
GLP:	Yes (certified laboratory)  Screening of confirming in water was not performed according to CLP

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the long-term effects of BYF 2960 (Sample description: TOX 08508-01 (Batch ID: 2009-000239) purity 26.2 % w/w) on development, reproductive capacity and behaviour of Daphnia magna.

Daphnia magna (<24 hour old geonates, 10 animals per study group) were exposed in a static-renewal system over a period of 21 days to rominal concentrations of 0.8, 1.6, 302, 6.4, 42.8 and 25.6 mg a.i./L. In addition, a water and softent control were tested. Test solutions were repewed in 48 hours (working days) respectively 72 hours (weekend intervals.

The accompanying chemical analysis of BYI 02960 in the freshly prepared test solutions at start of the chosen exposure intervals revealed recoveries between 102% and 109% (mean: 105%) of the corresponding nominal Concentrations. The corresponding concentrations of the aged test solutions at the end of the exposure intervals ranged between 102% and 117% (mean: 107%) of nominal. All results submitted by this coport are related to nominal test concentrations of the active ingredient.

Endpoints measured and recorded were the lotal living offspring per surviving parental animal, the parental age at first offspring emergence well as the rate of parental survivors and the survivors' body-length and dry body mass at the said of the study.

No mortalities were observed for any treatment level. However, for some of the other parameters detrimental effects could be observed.

The overall-NOFC was determined to be 3.2 mg a.i./L, based on a reduced parental body length at test termination. The corresponding LOEC is 6/4 mg a.i./L. The "Maximum Acceptable Toxicant Concentration" (MATC) calculated as geometric mean between NOEC and LOEC, is 4.5 mg a.i./L

### MATERIAL AND METHODS

### A. Materials

# 1. Test material

Test item: BYI 02960 Type of test material: Substance technical Chemical state and description: Beige powder Batch number: 2009-000239 Sample description: TOX 08508-01

2(5H)-Furanone, 4-[[(6-cMoro-3-pyriding)]methyl]( CAS name:

difuoroethyl)amino]

CAS#: 951659-40-8

al tvr IUPAC name: 4-[(6-chloropyriding

Purity: 96.2% w/w Storage conditions: +10 to +30

# 2. Test solutions

Dimetrylformamide ( Vehicle:

1000 L/L Concentration of vehicle:

Water and solvent control Controls:

Evidence of undissolved material: None

# 3. Test organisms

Species: Common name: Water flea

088) equal (6) type (4) (Baird 1991) Cenotype No. 2 Bradley Strain:

Dhouse Eulture Source:

Age at study initiation: 24 hour old neonates

vessel with 100 mL (corresponding to 1x108 cells/L) Maintenance of cultures

Temperatures

8 hours dark Photoperiod:

Food:

# B. Study design and method

1. In life dates

# 2. Design of biological test

Daphnia magna (<24 hour old neonates at test start) were exposed to BYI 02960; (purity 96.2% w/w) in a seroi-static system over a period of 21 days. Nominal concentrations were 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 mg a.i./L. In addition a water and solvent control were tested. The vessels were glass beakers (250 mL filled with 100 mL test medium). The test was conducted with 10 replicates of individually exposed animals per treatment.

# 3. Method of analytical weritication

For analytical verification of the test item concentrations samples were taken at day 0 (fresh), day 2 (aged), day 9 (fresh), day 12 (aged), day 19 (fresh) and day 21 (aged) from all concentrations. The LOD and LOQ were 0.02 μg/L and 0.05 μg/L, respectively. The range of linearity was 0.05 to 11 μg/L.

### 4. Observation and measurements

Immobilisation of daphnids, symptoms, data on reproduction and growth as well as physical-chemical. water parameters were assessed as indicated below in the result section.

# 5. Statistical analysis

Quantitative data like offspring counts and parental growth measurements were analysed on variance. homogeneity (Levene's Test) and normal distribution (Shapiro Wilk's Test) on a 5% level of significance.

Parametric procedures for homogeneous data involved subjecting reproduction data to standard ANOVA. If significant differences among the means were indicated multiple comparison procedures (e.g. Dunnett's multiple t-test procedure) or, in case of monotonous decrease of responses, adequate step down trend-tests (e.g. Williams multiple sequential t-test procedure) were performed on a 5%-level of significance ( $p \le 0.05$ ), to indicate which treatment groups differed significantly from the control. For non-parametric procedures the Mann-Whitney-Wilcoxoff U-test for independent samples was applicable, and alternatively multiple comparison procedure with Bonferroni-Correction was used.

Measurements of physical and chemical parameters of the test solutions are summarized as follows:

Test temperature:
pH:
Dissolved oxygen (mg/L):
Aeration used:
Photographic 13 to 15 German degrees (2H)
2 dH as carbonate hand Photoperiod: Hardness: Conductivity: AdH as carbonate hardness 488 to 563 upinos/cm

# B. Analytical Findings

Analytical verification of test solutions revealed measured concentrations of 102% to 109% (mean: 105%) in freshly prepared and 102% to 177% (mean: 107%) in aged test solutions of nominal. Thus, biological results are reported in nominal. Detailed malytical results are presented in the following

Table: Nominal and measured concentrations of BYI 02960

nominal		Measured concentrations (mg a.i./L)				9/- of o		
concentrations (mg a.i./L)	Medium	day 0	day 2	day 9	day 12	day 19	day21	% of ° nontinal
0.8	(fresh)	0.836		0.847		0.828 🕿	9	094 to 1170
0.8	(aged)		0.858		0.939	Ž	0.837	C 1170
1.6	(fresh)	1.66		1.69		1.640	~	102 6
1.0	(aged)		1.78		1.74	,A	1.63	Mí (
3.2	(fresh)	3.31		3.45		£3.47		%104 to
3.2	(aged)		3.4	W.	3.55	W"	<b>8</b> 32	L≫ 111 <i>00 i</i> * L
6.4	(fresh)	6.7		6.74	Ô	6.53	, Ø 3	102, to
0.4	(aged)		6.76	,Ű	6.74	a(	o 6.91♥	. Q08 A
12.8	(fresh)	13.2		3.9	4	å 13.6 Å	Å.	903 to 9
12.0	(aged)		14	<b>%</b>	13.6 ⋅	<b>O</b> ' <b>S</b>	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	) 11 <b>%</b>
25.6	(fresh)	27.5	<b>&amp;</b> ,	27 <sub>6</sub> 4°	13.6 ·	269		1 <b>05</b> to
23.0	(aged)		27.60	, O S	© 27. <b>3</b> ∜	~ ~ ~	≥ 27 <sub>6</sub> 1	<u>4</u> 108

# C. Biological Findings

Observations on immobilization, reproduction and growth and listed as follows

Effect of BYI 02960 of survival and reproduction of Daphua magna Table:

treatment level [mg a.i./L]	mean size of adult females [mm ± SD)	A	% adult sovival	offsøring per	parent age ( at 1st () offspring omergence [days]	% offspring dead or affected
water control	4.5 ± 9.2	\$0.97 <u>#</u> 0.1		7 126 ± 22 <b>%</b>	°>9.1	0
solvent control	4.6 0.1	<sup>™</sup> 0. <b>9</b> ©± 0.1 ©	<b>3</b> 00 2	$126.2 \pm 1796$	<b>№</b> 9.4	0
0.8	$45 \pm 0.3$	$97 \pm 0.2$	1000	32.8 ±31.1	<b>©</b> 9	0
1.6		√9.89 <del>&amp;</del> 0.2	100	♪122.3₽19.7 <i>/</i>	9.2	0
3.2	9 4.4 <b>3</b> 6.1	0.83 0.1 6	Ø \$100 S	$1212 \pm 21.9$	9.4	0
6.4	4.30 ± 0.2 <	$0.98 \pm 0.2$	100	109.7 ± 39.4	9.3	0
12.8	4.2 * ± Q.2	83 ± 0,55	0 100	@02.8*\div24.3	9.6	0
25.60	3.9 * ± 9.2	9.63 *±0.1	160	₹85.4 <del>*</del> 23.3	9.6	0

<sup>\*</sup> significant difference from solvent control by 5%;

No sublethal behavioura change

# D. Validity Coteria

Control mortality was less than number of live offspring per parent was greater than 60 in the control.

# E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

NOEC (overati): ′3.2 mag a.i./L NOEC (overage):

NOEC (improbilisation):

NOEC (time to first broad):

NOEC (hving no mates/adult):

NOEC (adult body length): ≥ **2**06 mg a.i./L  $\geq 25.6$  mg a.i./L 6.4 mg a.i./L 3.2 mg a.i./L NOFC (adult dry weight): 2 12.8 mg a.i./L

### **CONCLUSION**

The chronic effect of BYI 02960 on growth and reproduction of *Daphnia magna* can be quantified as an overall-NOEC of 3.2 mg a.i./L. The corresponding LOEC is 6.4 mg a.i./L. Therefore the "Maximum Acceptable Toxicant Concentration" (MATC), calculated as geometric mean between NOEC and LOEC, is 4.5 mg a.i./L (nominally).

Report:	KIIA 8.3.2.1/02; (2012)
Title:	Influence of BYI02960-succinamide (sech.) on development and reproductive output.
	of the waterflea Daphnia magna in a static-renewal deporatory test system
Report No:	EBRVP185
Document No:	M-424700-01-2
<b>Guidelines:</b>	OECD-Guideline No. 211
	EC Council Doculation No 440/2009 Mathed COO "I O S S S S
	U.S. FIFRA72-4 (1982) \( \tilde{\pi} \) \( \ti
	U.S. EPA- OPPTS Guideline \$50.1300
<b>Deviations:</b>	None None
GLP:	Yes (certified laboratory)
	Screening of condaminants in water was not performed according to GLE

## **EXECUTIVE SUMMARY**

The aim of the study was to determine the long-term effects of BY002960-succinamide Sample description: TOX 09343-01 (Batch 10: BC\$ CR74729-0001); purity 97.8 % www on development, reproductive capacity and behaviour of *Daphnig magny* during 21 days of exposure.

Daphnia magna (<24 hour old beonates, 10 replicates per treatment) were exposed in a static-renewal system over a period of 21 days to command concentrations 3.5.8.1, 18.7, 43.3 and 100 mg pure metabolite (p.m.)/E. In addition, a water control was rested. Lest medium renewal took place on Mondays, Wednesdays and Fridays, immediately after new test solutions had been prepared. The test was conducted with 10 replicates of individually exposed animals.

The chemical analysis of BYI02960-succinamide in the freshly prepared test solutions at start of the chosen exposure intervals revealed recoveries between 103% and 111% (mean: 106%) of the corresponding nominal concentrations. The corresponding concentrations of the aged test solutions at the end of the exposure intervals ranged between 104% and 509% (mean: 106%) of nominal. Based on analytical findings the piological endpoints are reported as nominal figures.

The endpoints measured and recorded were the total living offspring per surviving parental animal, the parental age at first offspring energence as well as the rate of parental survivors and the survivors' body-length and dry body mass at the end of the study. Although no mortalities occurred at any test level, effects on some of the other endpoints were recorded.

The overall-NOEC was determined to be 43.2 mg p.m./L based on an increased age at first reproduction at the highest test concentration of 100 mg/p.m./L. The corresponding LOEC is 100 mg p.m./L. The "Maximum Acceptable Toxicant Concentration" (MATC), calculated as geometric mean between NOEC and LOEC, is 65.8 mg/p.m./L (nominal).

### MATERIAL AND METHODS

### A. Materials

### 1. Test material

Test item: BYI 02960-succinamide (BCS-CR74729) Type of test material: Substance, technical (pure metabolite)

Chemical state and description: White powder Batch number: BCOO 6329-2-10 Batch ID: BCS-CR74729-01-01 Sample description: TOX 09343-01

IUPAC name: 4-{[(6-chloropyridin-3-yl)methyl](2

acid

Purity: 97.8% w/w Expiration date: 2012-03-13 Storage conditions: +10 to +30 °

# 2. Test solutions

Vehicle: None Controls: Evidence of undissolved material:

## 3. Test organisms

Species: Common name:

(Bradley 988) Qual to type B (Baird 1991) Strain:

Source: nhouse-culture

Age at study initiation

per test vessel with 100 mL (confesponding to 1x108 cells/L) Feeding during text

Maintenance of culture Temperature:

Photoperiod:

# B. Study design and met

Daphnia magna (<24 hour old neonates at test spart) were exposed to BYI 02960-succinamide; (purity 97.8 % www) in a static renewal system over period of 21 days. Renewals took place on days 2, 5, 7, 9, 12, 14, 16 and 19, Nominal concentrations were 3.5, 8.1, 18.7, 43.3 and 100 mg pure metabolite/L. In addition a water control was tested. The vessel owere glass beakers (250 mL filled with 100 mL test medium). The test was conducted with 10 replicates with one daphnid each.

# 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at day 0 (fresh), day 2 (aged), day 9 (fresh), day 12 (aged), day 19 (fresh) and day 21 (aged) from all concentrations. The LOQ was  $10.9 \mu g$ . The range of linearity was 10.9 to 2180  $\mu g/L$ .

# 4. Observation and measurements

Immobilisation of daphnids, symptoms of intoxication, data on reproduction and growth as well as physical-chemical water parameters were assessed as indicated below in the result section.

## 5. Statistical analysis

Quantitative data like offspring counts and parental growth measurements were analysed on variance homogeneity (e.g. Bartlett's Test) and normal distribution (e.g. Kolmogorroff-Smirnov Test) on a level of significance using the treatment levels and untreated control as co-variates.

Parametric procedures for homogeneous data involved subjecting reproduction data to standard ANOVA. If significant differences among the means were indicated, multiple comparison procedure (e.g. Dunnett's multiple t-test procedure or, in case of monotonous decrease of responses adequate steps down trend-tests (e.g. Williams multiple sequential t-test procedure) were performed on a 5% revel of significance (p  $\leq$  0.05), to indicate which treatment groups differed significantly from the control  $\mathcal{L}$ non-parametric procedures the Mann-Whitney-Wilcoxon U-test for independent samples was applicable, and alternatively multiple comparison procedure with Bonferoni-Correction was assed

# RESULTS AND DISCUSSION

# A. Physical and Chemical Parameters

A Physical and Chamical Parameters
Measurements of physical and chemical parameters of the test solutions are sommarized as follows.
Test temperature:  pH:  Dissolved oxygen (mg/L):  A cryption used:  20 4 to 24 8 °C  8.5 to 9.1
pH: OF to & V S S S S S S
Test temperature:  pH:  Dissolved oxygen (mg/L):  Aeration used:  Photoperiod:  248 to 248 ° C  8.5 to 9.1  8.5 to 9.1  46 hrs light, 8 hr@dark
Aeration used:
Aeration used:  Photoperiod:  Hardness:  14 Gerban degrees (dH)
Hardness: 14 Geman degrees (dH)
Alkalinity: 3 dH as carbonate hardness
Conductivity: 62720 633 mhos cm
Conductivity: 62 Mo 63 Symhostem 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
B. Analytical Findings
Analytical verification of Oct solutions of real American and collectivities of 03% to 111% (mean

Analytical verification of lest solutions revealed measured concentrations of 103% to 111% (mean: 106%) of nominal in freshly prepared and 100% to 109% (mean; 106%) of nominal in aged test solutions. Biological results are reported as nominal. Desailed analytical results are presented in the following table:

Nominal and ancasured concentrations of BYT 02960, succinamide Table:

			() 4	,	2			
Nominal			ຶ∕> Meassyl	red concent	ration (mg	p.m./L)		% of
concentrations (mg p.m./L)	medium	Jay 0	day 2	Say 9	))*	day 19	day21	nominal
3.5 4	(fresh)	3.25		3. <b>T</b>		3.72		104 to
	(aged)	Š	3.7%		3.66		3.71	108
8.1	(fresh)	8.67		© <sup>7</sup> 8.63		8.59		106 to
0.1	(avged)		8.69	Ý	8.63		8.56	107
18.7	(fresh)	J9.6		19.4		19.8		103 to
10.7	(aged)		2 <b>Q</b> 0		19.6		19.8	106
43.3	(firesh)	47.3	_@/	48.1		44.7		103 to
م الم	(aged)		<b>47.0</b>		45.3		45.2	111
160		Õt06		105		103		103 to
190	(aged)	Ş	107		107		108	108

# C. Biological Findings

Observations on immobilization, reproduction and growth are listed as follows:

Table: Effect of BYI 02960-succinamide on survival and reproduction of *Daphnia magna* 

Treatment	parental endpoints			r	eproductive endpo	oints
mg pure metabolite/L (nominally)	body length (mm ± SD)	dry body mass (mg ± SD)	survival (%)	total offspring per parent animal (n ± SD)	parent age at first offspring emergence (days ± SD)	neonates (*) (% of total) offspriog affected (*) dead(*)
control	$4.3 \pm 0.2$	$0.97 \pm 0.2$	100	$104.9 \pm 16.0$	$9.0 \pm 0.0$	
3.5	$4.2 \pm 0.1$	$0.86 \pm 0.2$	100	$101.9 \pm 12.5$	9.1 ±0.3	00 50 0
8.1	$4.2 \pm 0.2$	$0.90 \pm 0.2$	100	$92.7 \pm 18.8$	9.2€0.4	
18.7	$4.2 \pm 0.1$	$0.91 \pm 0.2$	100	90.0 ± 5.4	$90 \pm 0.0$	80 N 90
43.3	$4.3 \pm 0.0$	$0.83 \pm 0.2$	100	98.5 ± 8.8	$0.0 \pm 0.0$	
100	$4.2 \pm 0.2$	$0.90 \pm 0.2$	100	$906 \pm 31.7$	\$9.7 *± 1.9 0	

<sup>\*</sup> statistically significant difference from untreated control (William's t-test, p < 05, or sided)

No sublethal behavioural changes were observed

# D. Validity Criteria

All validity criteria were met. No mortality occurred in the control. Mean number of live offspring per parent was greater than 60 in the control.

# E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived

NOEC (immobilisation):	
NOEC (time to first brood):	43.3 mg pure metabolite/L
NOEC (living neonates/adult);	100 mg pure metabolice/L
NOEC (adult body length):	100 mg pure metabolite/L
NOEC (adult dry weight);	100 mg pare metabolite
NOEC (overall):	43.3 mg pure metabolite/I

### CONCLUSION

The chronic effect of RVI 02000-succinantide on growth and reproduction of *Daphnia magna* can be quantified as an overall-NOEC of \$3.3 mg pure inetabolite/L. This NOEC is based on an increased age at first reproduction at the highest test concentration of 100 mg pure metabolite/L. The corresponding LOEC is 100 mg pure metabolite/L. The "Maximum Acceptable Foxicant Concentration" (MATC), calculated as geometric mean between NOEC and LOEC, is 65.8 mg pure metabolite/L (nominal).

# IIA 8.3.2.2 Chronic toxicity for tepresentative species of aquatic insects

From the disecticidal activity of BYI 02960 aspecific activity against aquatic insects may be anticipated. To address the chronic toxicity of the insecticide, the growth and development test for *Chironomus riparius* (OEOD 219) was undertaken. Chronic tests on the midge were also performed with the soil metabolities 6-CNA and OFA following the rationale described earlier considering the major structural differences to the parent compound.

As previously noted DFA is a very strong acid that cannot be handled as the free acid, it was therefore tested as the social with the knowledge that dissociation will occur in the aquatic system.

Report:	KIIA 8.3.2.2/01 (2011)
Title:	Chironomus riparius 28-day chronic toxicity test with BYI 02960 (tech.) in a water sediment system using spiked water
Report No:	EBRVP025
Document No:	M-401792-01-2
<b>Guidelines:</b>	OECD Guideline 219
Deviations:	None O' O' O'
GLP:	Yes (certified laboratory)
	Screening of contaminants in water was not performed according to GQ

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the effects of BYI 02960 Sample description: TOX 08508 00 (Batch ID: 2009-000239); purity 96.2% w/w) of emergence and development of *Garronomus riparius*.

Midge larvae of *Chironomus riparius* (1st instar larvae, 2-3 days old, 4 replicates of 20 per treatment and control) were exposed in a static water sediment system (spiked-water exposure) over a period of 28 days to nominal concentrations of 1.25, 2.50, 5.00, 10.0, 20.0 and 40.0 gg a.i. (1). In addition a water control and solvent control were tested. Four replicates per concentration and control with 20 animals each were tested.

Recoveries of active substance were measured three times during the study: 1 hour, 7 days and 28 days after application in one additional test confidence of each nominal initial test confernation.

Chemical analysis of overlying water and pore water over time reflect expected agostic fate data with high recoveries of 85 % to 110% (mean 99 %) at the beginning of the exposure period in the overlying water of all test concentrations.

On day 7 recoveries from 97 % to 83 % (mean 60 %) were found. Recoveries from 30 % to 52 % (mean 41 %) of noming test concentrations were found on day 28.

Chemical analysis of the pore water over time yield 0.6% of nominal concentration on day 0, 2.0 % on day 7 and 22 %, on day 8.

Initial nominal concentrations were used for reporting and evaluation of the results. Additionally, the results were calculated to initial measured concentrations.

Emergence, sex and development rates were determined.

The start of emergence was reduced for one day at test concentration of 20.0 µg a.i./L. No emergence was observed at the highest test concentration of 40 0 µg a.i./L. The overall NOEC was determined to be 10.0 µg a.i./L (initial measured: 40.5 µg a.i./L based on emergence rate and development rate.

# MATERIAL AND METHODS

# A. Materials

1. Test material

Test item: BYI 02960 Type of test material: Substance, technical Chemical state and description: Beige powder 102000022313 Specification No.:

2009-000239 (Batch code: ANI 02960-0 Batch number:

Sample description: TOX 08508-00 CAS#:

**IUPAC Name:** 

Purity:

Stability:

2. Test solutions

951659-40-8
4-[(6-chloropyridin-3%/lmethyl)(2,2-drilluoroethyl)araino]furan-2(549)-one %
96.2% w/w
Expiry date: 16 Jan 2011, when stored at +25 ± 5°C (storage conditions from +2°C to \*30°C are also acceptable)

Dinaethyltonamide (DMF)
Water control and solven control

Chironopius riparius
Midge
University of am
Inhouse-culture since 2006
Ast instar larvae (2-3 days old) Vehicle: Controls:

3. Test organisms

Species:

Common name:

Strain:

st instar larvay (2-3 days old) Source: Age at study initiation:

Maintenance of culture

Temperature:

Photoperiod: A6 how Plight, Shours dark

20 ± 2°C

30 hour light shours dark

Green algae and an addeous suspension of a plant material based fish Food:

B. Study design and methods

Chironomus ciparius first instar lavae were exposed 18 BYI 02960 (purity 96.2 % w/w) in an artificial water-sediment system over a period of 28 days. Nominal concentrations were 1.25, 2.50, 5.00, 10.0, 20.0 and 40.0 µg a.i. I. In addition water control was described when were tested. The vessels were glass beakers (0.6 L) filled with 1.5 cm layer of artificial sediment and a 6.0 cm layer of water (M7medium). The test was conducted with 4 repocates per treatment, 4 replicates for the water control and 4 for the solvent control

sediment laver: 1.5 cm 75.80% quartz sand sphaguum mos peat 4% kaokinite 20% calcium cobonate 0.20%

## 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at day 0 (1 hour), day 7 and day 28 from all concentrations. BYI 02960 (purity 96.2 % w/w) served as analytical standard. The LOQ was 0.115 µg a.i./L.

# 4. Observation and measurements

Emergence of midges and development rates as well as physical-chemical water parameters were assessed as indicated below in the result section. The test vessels were observed at least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times per week to make a visual assessment of any least three times the times three times thr week to make a visual assessment of any behavioural differences compared to the control The sex, time point of emergence and number of emerged midges was recorded daily during the period of emergence.

# 5. Statistical analysis

The statistically different distribution between sexes compared to the assumption of 50% females and 50% males are judged by a  $\chi^2$ -r x 2 table test. EC values (e.g.  $\chi=15.50$ ) and confidence intervals after 28 days were calculated by probit (or logit, webit, etc.) analysis or an case of fail we by son parametricmethods from the appropriate parameters (endpoints). The LOES determinations from the appropriate parameters (endpoints) were done, using the ANOVA procedure (α #0.05 one sided) and properly selected multiple t-tests. In case of a limit test (comparison of control and one treatment group only) the Student t-test can be used. Statistical evaluations were done using the commercial program ToxRat Professional.

# RESULTS AND DISCUSSIC

# A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test solutions are summarized as follows:

Test temperature
pH:
Dissolved daygen (mg/L): 750to 8.5
Dissolved oxygen (% saturation 9) 84.2 % (for 7.5 mg/L)
Aeration used:
Photoperiod: 2 164bours light; 8 hours date
Light intensity: 500 to 1000 Lux
Hardness: 26/19/mg/L as CaCO <sub>3</sub> )
Alkalinity: 1246 to 23 4 mg/b as CaCO <sub>3</sub> )
Sediment part. 60 5 60 5
Sediment water content: 31.50%
Sediment organic carbon content 2%

# B. Analytical Findings

Chemical analysis of overlying water are pore water over time reflect expected aquatic fate data with high recoveries of 85 % to 110 (mean 99 %) at the beginning of the exposure period in the overlying water of all test concentrations. On day 7, recoveries from 37 % to 83 % (mean 60 %) were found. Recoveries from 30% to 52% (mean 41%) of nominal test concentrations were found on day 28. Chemical malysis of the pore water over time yield 0.6 % of nominal concentration on day 0, 2.0% on day 7 and 2.2%, on day 28. Biological results are reported as mean measured. Detailed analytical results are presented in the following table:



Table: Nominal and measured concentrations of BYI 02960

	analytical results of BYI 02960, means of two analyses each [μg a.i./L]					
initial nominal	day 0	(1 hour)		day 7		day 28 👋 🛮 🕜
concentration	analysed	% of nominal	analysed	% of nominal	analysed	% օք©ոնաinթվ
[µg a.i./L])			over	lying water		* . Ç
control	< 0.1115		< 0.1115		< 0.1115	\$ \$ \frac{1}{2}                                                                                                                                                                                                                                  \text
solvent control	< 0.1115		< 0.1115	Į.	$^{9}$ < 0.1115 $^{\circ}$	(M)
1.25	1.22	98	0.671	3 54 J	0.382	31 30 4 4
2.5	2.13	85	1.23	49 Q	0.73	30 🗸
5.0	4.41	88	1.83	37 <sup>©</sup> '	1.82	Q 36 V
10.0	10.5	105	6331	<b>√</b> Q <sup>y</sup> <sub>∕~</sub> ∘	4,76 s	(%)O ((//)
20.0	21.3	107	<b>20</b> 6.5	83 0	<b>₹</b> 0.3, ○	& 52 W
40.0	43.8	110	29.7 。	. ™ 74.~	⊚ 20. <b>%</b>	52 55
average %		99	Y . Û	©water o	ľ Š	41
			<b>⊘</b> por	ewater 8		Y A L.
control	< 0.1115		≪9.111⁄5		\$0.1115°	
solvent control	< 0.1115	\$	× 0.1118	0, 7, ,	<b>€</b> 0.1 <b>5</b> ¥5	\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sqrt{2}}\$\frac{1}{\sq
1.25	0.214	8 4	0.283	¥ 19 ×	03	Ç OŠ
2.5	0.184	00.5 V	- N	7 0.3	\$524	<u>ۇ</u> 1.4
5.0	0.314	° U.4	0.838	5 <sup>7</sup> 1 0	© 1.3300°	° 1.9
10.0	0.688	0,50	♀ 3.33ǰ	1 O) 2. <b>3</b> . ~	) 4.HV	2.9
20.0	2.32	×0.7	9662	<i>29</i>	<b>8</b> 095	<b>∀</b> 2.6
40.0	4.62 ∜		19.2	3.0	© 16.6	2.8
average %	\$	0.65	W W	2.0	ŊŢ	2.2

<sup>1) =</sup> calculated to the real volume of pore water and the applied amount of a.i.

# C. Biological Findings

Start of emergence was on day 13 and 14 for the controls and test concentrations from 1.25 to 10.0 µg a.i. /L. The start of emergence was reduced for one day at test concentration of 20.0 µg a.i./L. No emergence was observed at the highest test concentration of 40.0 µg a.i. /L. Emergence and development rates were observed as follows:

Table: Effect of BYL 02960 on survival and reproduction of Chironomus riparius

Initial nominal	number of	<b>Vemerge</b> nc	e of Introduc	ed larvae	devel	opment rate	(1/d)
test	emerged		(pooled sex)	Ş			
concentration	anidges O	total %	mæle %	female %	pooled sex	male	female
(μg a.i./L)	(introduced)			ď			
Controls (pooled)	144 (160)	90%	×41.3 «	48.8	0.058	0.064	0.052
1.25	<b>70</b> (80) $^{\circ}$	87.5	\$ 42. <b>5</b> \%	45	0.058	0.064	0.053
2.5	<b>₹</b> 74 (80 <b>)</b>	92.5 Ô	42,3	50	0.057	0.064	0.051
5	67 (80)	≥> 83.8Q	<b>41.3</b>	42.5	0.058	0.065	0.052
10	§ 71 (80) 🔏	<i>9</i> 88 <b>⊗</b>	<b>41.3</b>	47.5	0.059	0.065	0.054
20	42* (80)	<b>3</b> 2.5	√ 18.8	33.8	0.050*	0.058*	0.044*
40	<b>≫</b> Ø (80 <b>)</b>	- 0	-	ı	-	=	-

<sup>\*</sup> significant difference ( $\alpha = 0.05$ )

The Exact r-x2 Table Test indicates no statistically different distribution between sexes compared to the assumption of 50% females and 50% males. Therefore males and female were pooled for all further endpoint valculations to increase the statistical power.

A statistical significant ( $\alpha$  = 0.05) effect on emergence rate was evaluated for 20.0  $\mu$ g a.i./L, resulting in an NOEC of 10.0  $\mu$ g a.i./L.

For the development rate (pooled sex) a statistical significance was evaluated at the highest test concentration with emergence of 20.0  $\mu$ g a.i./L, resulting in an NOEC of 10.0  $\mu$ g a.i./L

# D. Validity Criteria

Mean number of live offspring per parent was greater than 60 in the control. The emergence in the control(s) was >70% of introduced larvae at the end of the est. The emergence was between 2 and 25 days after their introduction into the control vessels. The oxygen content in the water body was > 60% of saturation at the end of the test in all test vessels. The pH of the overlying water was between and 9 in all test vessels. The water temperature did not to offfer by more than \$1°C over the whole exposure period.

# E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived;

# Endpoints based on nominal concentrations.

Endpoints	NOLE LOEC EGE [µg a.i./L] [µg a.i./L] [µg a.i./L]	©EC <sub>50</sub> © [μg a.i./L]
emergence rate (pooled sex) (95 % confidence limits)	20.0 V 13.4 V 19-15.2)	20.3 (185 – 22.2)
development rate (pooled sex) (95 % confidence limits)	20.00	32.6 (27.9 – 48.7)

# Endpoints based on initial measured concentrations:

	V// _ a`	87 A	J*\ <u>\</u>		
Ī	Endpoints O	1 WELC	LOEC [µg/a.i./LD	EC15	EC50
L	₹ . \ \	<sub>ω</sub> [μg a.i./L]	_ [µg∕a.i./L⊅°	Çag a.i.∕L	[µg a.i./L]
Ī	emergence tate (posed sex)	165	W 21 %	14.3	21.8
	(95 % confidence limits)			0 (12,6 16.1)	(19.8 - 23.8)
	development rate (pooled sex)	2 5 0°	21.2	©21.9	35.3
	(95% confidence limits)	₩ Y0.5	21.3	(24.2 - 23.1)	(30.2 - 51.8)

# CONCLUSION

The chronic effect of (BCI 02960) on development and emergence of *Chironomus riparius* can be quantified as an overall-NQEC of 10.00 µg a.i./L (initial measured: 10.5 µg a.i./L).

Report: KIIA 8.3.2.2/02; (2011)	
Title: Chaphomus riparios 28-day chronic toxicity test with Sodium of	lifluoroacetate in a
Title Chiconomy Pipario 28-day chronic toxicity test with Sodium of water-sediment system using spiked water – limit test	
Report No:	
Document No. 2 M-48913-01-2 @	
Guideline No. 219	
Deviations: V None O	
GLP: Yes (certified laboratory)	
GLP: Yes (certified laboratory)  Screening of contaminants in water was not performed acco	rding to GLP.

# EXECUTIVE SUMMARY

The aim of the study was to determine the effects of sodium difluoroacetate (Na-salt of BYI 02960-DFA) (Sample description: TOX 08988-01 (Batch ID: 2009-000239), purity > 99.0% w/w) on emergence and development of *Chironomus riparius*.

Midge larvae of *Chironomus riparius* (1st instar larvae; 2-3 days old, 20 per replicate with 6 replicates) © were exposed in a static water-sediment system (spiked-water exposure) over a period of 28 days to a nominal concentration of 100 mg pure metabolite/L. In addition a water control was tested.

Recoveries of the metabolite were measured three times during the study: Khour, 7 days and 28 Car after application in one additional test container of the limit test concentration of 100 mg p.m. L and controls of the overlying water and the pore water of the sediment.

Chemical analysis of sodium difluoroacetate in the overlying water over time showed high recoveries of 105% at the beginning of the exposure period of the limit test concentration. On day and 28 the determined amount of sodium difluoroacetate was 95.1% and 98.3% respectively.

Chemical analysis of the pore water over time yield 55% of none and on day 0, 6.61% on day and contains a second of the pore water over time yield 55% of none and on day 0, 6.61% on day and contains a second of the pore water over time yield 55% of none and one day 0, 6.61% on day and contains a second of the pore water over time yield 55% of none and one day 0, 6.61% on day and contains a second of the pore water over time yield 55% of none and one day 0, 6.61% on day and contains a second of the pore water over time yield 55% of none and one day 0, 6.61% on day and contains a second of the pore water over time yield 55% of none day 0, 6.61% on day and contains a second of the pore water over time yield 55% of none day 0, 6.61% on day and contains a second of the pore water over time yield 55% of none day 0, 6.61% on day and contains a second of the pore water over time yield 55% of none day 0, 6.61% on day and contains a second of the pore water over time yield 55% of none day 0, 6.61% on day 20% of the pore water over time yield 55% of the pore water over the pore wa 7.95%, on day 28.

The initial nominal limit concentration was used for reporting and evaluation of the results. The initial nominal limit concentration was used for reporting and system. Emergence, sex and development rates were determined. The overall-NOEC was determined to be the limit dose tested, ≥ 100 mg pure metabolite/L.

4	<b>TD</b> .	
	Lest	material
1.	1050	material

Test item:

Type of test material;

Chemical state and a Batch ID:

Batch No.

apr 2011, to be stored at +10 to +30°C

# 2. Test solution

# 3. Test/organisms



Country)

2.3 days old)

3.4 light, 8 hours dark

3.4 light, 8 hours dark

3.5 light, 8 hours dark

3.6 light, 8 hours dark

3.6 light, 8 hours dark

3.7 light, 8 hours dark

3.8 light, 8 hours dark

3.9 light, 8 hours dark

3.9 light, 8 hours dark

3.0 light, 8 hours dark

3.1 light, 8 hours da

fisher, the state of the state

# B. Study design and methods

1. In life dates 18 Jan to 22 Feb, 2011

# 2. Design of biological test

Chironomus riparius first instar larvae were exposed to sodium difluoroacetate. Na-salt of BYI 0296 DFA) (code: BCS-AB60481; purity > 99.0 % w/w) in an artificial static water-sediment system (spiked & water) over a period of 28 days. The nominal concentration was 100 mg pure metabolite. In addition a water control was tested. The vessels were glass beakers 0.6 L; diameter 9.5 cm) filled with 1.5 cm layer of sediment and a 6.0 cm layer of M7 test medium. The test was conducted with 20 larvae por replicate (6 replicates). During the study the larvae were fed at least about three times per week with a commercial ornamental fish food extract (trade name Tetra Phyll) as used for the breeding. An appropriate amount of this suspension (about 0 %- 1 mg/retraphyll darvac day) was added to each test container.

Sediment:
sediment layer:
quartz sand
sphagnum moss peat
kaolinite
calcium carbonate

1.5 cm
20%
20%
3. Method of analytical verification

sediment layer:	1.5 cm
quartz sand	75.0%
sphagnum moss peat	4%
kaolinite	20%
calcium carbonate	0.1%

3. Method of analytical verification of the test item concentrations samples were taken at day 0 (1 hour), day 7 and day 28 from all concentrations. The test substance set ved as analytical standard. HPLC-MS/MS was used as analytical method. The limit of quantification (LOO) was 1.048 fug/L.

# 4. Observation and measurements

The test vessels were also observed at least three times per week to make a visual assessment of any behavioural differences compared to the control. The sex, time point of emergence and number of emerged midges was recorded daily during the period of emergence. As only fully emerged adults are relevant for the endpoints of this study, larvae which did not yet mature were not taken into account for emergence rates and development time. To determine number and sex of emerged adults, the covering plates of each test container were carefully proved and the midges, which mostly stayed at the sides of the vessels, were enumerated; after identification of the sex (male midges have feathered antennae) midges were removed. Emergence of midges and development rates as well as physical-chemical water parameters were assessed as indicated below in the result section.

# 5. Statistical analysis

The statistically different distribution between sexes compared to the assumption of 50% females and 50% males are judged by a  $x^2$ -r x 2 table set. EC<sub>x</sub> values (e.g. x = 15, 50) and confidence intervals after 28 days were calculated by probal (or logit, weibit, etc.) analysis or in case of failure by non-parametricmethods from the appropriate parameters (endpoints). The LOEC determinations from the appropriate parameters (endpoints) were done, using the ANOVA procedure ( $\alpha = 0.05$ , one sided) and selected multiple t-lests. In case of a limit test (comparison of control and one treatment group only) the Student t-test can be used. Statistical evaluations were performed using the commercial program ToxRat Professional.



### RESULTS AND DISCUSSION

# A. Physical and Chemical Parameters

RESULTS AND							
A. Physical and Companies of Measurements of Measurements of Measurements of Measurements of Measurements of Measurements of Dissolved oxygen Dissolved oxygen Aeration used: Photoperiod: Light intensity: Hardness: Alkalinity: Sediment pH: Sediment water consediment organic of Measurement of Measurements of Measur	Chemical Param	eters					
Measurements of	nhysical and cher	nical narar	meters of the to	est solutions ar	e summarized	as follows	Ö
Wicusarements of	physical and ener	neur purur	include of the to	est solutions ui	& Sammarized		
					Ď		
Test temperature:		20.3°C to	20.5°C		O <sup>T</sup>	A Z	
pH:		8.2 to 8.5	5		A		Ò
Dissolved oxygen	(mg/L):	7.7 to 8.8	B mg/L	`			J.
Dissolved oxygen	(% saturation ):	85.8 % (a	at 7.7 mg/L)	į į	, _		
Aeration used:		yes	¥.	ő	Q		, Ő <sup>Y</sup>
Photoperiod:		16 hours	light; 8 hours d	ark 🛴			
Light intensity:		852 Lux		, Y			,
Hardness:		302.6 to 3	320.40png/L as 0	CaCQ>> ~ ~ ~			
Alkalinity:		106.8 to 2	21&.6 mg/L <sub>g</sub> ås (	CaCG3) \( \sigma'' \)			
Sediment pH:		6.7		Ö 8			
Sediment water co	ntent:	32.6 %		, Q, ,			
Sediment organic of	carbon content	2.1			, O' &	4, 29	
		<i>\\</i> \\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\					
B. Analytical Fin	ıdings		y .			j.	
Chamical analysis	a of Codium diffus	2	· /			√)° ~1°~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Chemical analysis	s of Sodium diffuc	roacegate	in upe overlyin	ig Mater Over ti	me snowed ni	gn recoveries	
of 105 % at the be	eginning of the ex	posure per	nod of the limi	t test concentra	ation. Of day	%and 28 the	
determined amoun	nt of Sodium difly	oroacetale	g was \$95.1 %	ınd 98⁄3 %, res	pectively.		
Chemical analysis	s of the pore water	over	e y@ld 1.555 %	of nominal on	đấy 0,651 %	on day 7 and	
7.95 %, on day 28	B. Biological resul	ts are repo	Red asonitial	ominal/conce	ntrations. Deta	iled analytical	
results are present			- \$ .L'		4,	-	
*	aland measured co		hanf Resarr	5090 <b>8</b> 1 🗳 .	<b>W</b>		
		<b>9</b> .	7 2	%	<b>Y</b>		
			////	n diflueroacetat		. 20	
Nominal	<b>∂</b> da <b>y 0</b> (1 hc	ur) % <b>AT</b>	The state of the s	1y 7 <sup>©</sup>	day	7 28 % of	
concentration	anal tical	70 py. 7 nominal	anatytical	nominal	analytical	nominal	
(mg p.m./L)		, R	overlyi	ng wister	anaryticar	nommai	
control	\$\text{LOQ}\$	A NOT	LOO		< LOQ		
100	6 10 <b>5</b>	105	95.1	95.1	98.3	98.3	
(Mn)				water 1)			
control 🔷	©LOQ	Y Y	`≯LOQ		< LOQ		
100	20.0	1.55	6 10 kg	6.61	104	7.95	
1) Coloulato At the	real volteme of for	wolld nor	POOR VACCON				

<sup>1)</sup> Calculate (to to the real volume of per water per text vessely

# C. Biological Findings

Start of emergen was on day 1/3 and 4/4 for the controls and the limit test concentration of 100 mg p.m./L 95.8% of the inserted (n=120) larvae maturated to adults in the controls after 28 days. Observations on emergence and development rate are listed as follows:

Table: Effect of BCS-AB60481 on survival and reproduction of Chironomus riparius

Nominal	Number of	Emergen	Development rate		
concentration	emerged midges	total %	male %	female %	(1/d); pooled sex
(mg p.@."/L)	(introduced)				
Control	115 (120)	95.8	50.8	45.0	0.062
100	108 (120)	90.0	48.3	41.7	0.063

The Chi<sup>2</sup>-Test indicates no statistically different distribution between sexes compared to the assumption of 50% females and 50% males. Therefore male and female results were pooled for further statistical analyses to increase the statistical power. There was no statistically significant difference in emergence between the control and solvent control and at the limit test concentrations of 100 mg p.m./L as compared to the control findings. For the development rate (pooled sex) there was no statistical significant difference as compared to the control for the limit test concentration.

# D. Validity Criteria

Mean number of live offspring per parent was greater than 50 in the control. The emergence in the control(s) was >70% of introduced larvae at the end of the test. The emergence was between 12 and 23 days after their introduction into the control vessels. The oxygen content in the water body was > 60% of saturation at the end of the test in all test vessels. The pH of the overlying water was between 6 and 9 in all test vessels. The water temperature did not to differ by more than ± 1°C over the whole exposure period.

# E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived

		Sodium difluç	roacetate	mg pon./L]	
Endpoints *	Ø	NOEC NOEC		<b>₽</b> ØEC Ĉ	,
emergence rate (pooled sex)		2 100°		√> 10 <b>0</b>	~ C
development rate (pooled sex)		≥ 1000		√2 > 100	Ö

<sup>\*</sup> Based on nominal initial concentrations

# **CONCLUSION**

The chronic effect of sodium difluer acetae (Na-salt of EVI 02960-DIO); BCS-AB60481) on emergence and development of *Chironorulis riparius* can be quantified as an overall-NOEC, based on emergence and development of \$\geq 100\$ tog pure metabolite/L the concentration tested.

Report:	KINA 8.3,202/03; (2011)
Title:	Chironomus riparius 28-day chronic toxicity test with 6-Chloronicotinic acid in a
	Water-seriment@ystencusing stiked water – limit test
Report No.:	EBR P183
Document No.:	M216604-02-2 7 5 7
Guidelines: 🔷 💍	OECD Guideline 219
Deviations:	ONone O
GLP:	Yes (certified laboratory)
	Screening of contaminators in water was not performed according to GLP

### EXECUTIVE SUMMARY

each.

The aim of the study was to determine the effects of 6-Chloronicotinic acid (Sample description: AZ 16813 (code: AE F161089; purity 98.8%) on emergence and development *Chironomus riparius*. Midge lacoae of *Chironomus riparius* (1st instar larvae; 2-3 days old, 20 per replicate with 6 replicates) were exposed in a static water-sediment system (spiked water exposure) over a period of 28 days to a nonunal concentration of 100 mg pure metabolite/L (limit test). In addition, a water and a solvent control were tested. The study was conducted with six replicates per treatment level with 20 animals

Recoveries of the metabolite were measured three times during the study: 1 hour, 7 days and 28 days after application in one additional test container of the limit test concentration of 100 mg p.m./L and controls of the overlying water and the pore water of the sediment.

Chemical analysis of overlying water and pore water over time showed high recoveries of 102% at the beginning of the exposure period in the overlying water of the limit test concentration. On day the determined amount of 6-Chloronicotinic acid was 89.6% and 90.6%, respectively.

Chemical analysis of the pore water over time yielded 1.29% of nominal oxiday 0, 6.66% 7.29%, on day 28, respectively.

The initial nominal limit concentration was used for reporting and evaluation of the

Emergence, sex ratio and development rates were determined.

There was no statistical significant difference for emergence and development rate between the controls and the limit test concentration of 100 mg n m

based on the omi Therefore, the overall-NOEC was determined to be concentration.

### **MATERIAL AND METHODS**

### A. Materials

### 1. Test material

Substance, technical (bure metabolite)
Beige powder Test item:

Type of test material:

Chemical state and description

Origin Batch No.:

Batch Code:

Sample description: Purity:

Stability:

# 2. Test solutions

Vehicle: Controls:

# 3. Test organisms

Species: Common name:

University of (Germany)

Strain: Inhouse-curore since 2006 Source

1st instardarvae (2-3 days old) Age at study initiation: 5 to 1 Pag Tetraphyll per test vessel at least three times per week

Maintenance of Culture,
Temperature

Photoperiod: 16 hours light, 8 hours dark

Green algae and an aqueous suspension of a plant material based fish

food (Tetra Phyll®).

### B. Study design and methods

1. In-life dates January 18 to February 22, 2011

### 2. Design of biological test

Chironomus riparius first instar larvae were exposed to 6-Chloronicotinic acid (code:

AE F161089 00 1B99 0001; purity 98.8 %) in a static water-sediment system (spiked water) over period of 28 days. The nominal concentration was 100 mg pure metabolite/La

The test was conducted with 6 replicates. In addition a water and a solvent control were tested. The vessels were glass beakers (0.6 L; diameter 9.5 cm) filled with 1.5 cm layer of sediment and 0.6.0 cm layer of M7 test medium. The test was conducted with a larvae per replicate (6 replicates) During the study the larvae were fed at least about three times per week with a commercial framental fish food. extract (trade name Tetra Phyll®) as used for the breeding. An appropriate amount of this suspension

extract (trade name Tetra Phyll®) as used for the breeding. An appropriate amount of this suspension (about 0.5 - 1 mg Tetraphyll® /Larvae/day) was added to each jest container.

Sediment:
sediment layer:
quartz sand
sphagnum moss peat
kaolinite
20%
calcium carbonate

1.0%

3. Method of analytical verification

3. Method of analytical verification of test item concentrations samples were taken at say 0 (1 hour), day 7 and day 28 from all treatment levels. The test substance served as analytical standard. HPLC-MS/MS was used as analytical method. The limit of quantification (LQQ) was 1.145 µg/L.

# 4. Observation and measurements

The test vessels were observed at least three times per week to make a visual assessment of any behavioural differences compared to the control. The sex, the point of emergence and number of emerged midges was recorded daily during the period of emergence. As only fully emerged adults are relevant for the endowints of this study, larvae which do not yet mature were not taken into account for emergence rates and development time. To determine number and sex of emerged adults, the covering plates of each test container were carefully moved and the midges, which mostly stayed at the sides of the vessels, were enumerated; after identification of the sex (male midges have feathered antennae) midges were removed.

Physical-chemical water parameters were assessed as indicated below in the result section.

Sex ratio: The statistically different distribution between sexes compared to the assumption of 50% females and 50% males are judged by a 2-r x 2 table test. In case of emergence rate (no indications of statistical difference) male and female results were pooled for analyses to increase the statistical power in case of developmental rate statistical analyses were done separately for each sex.

The TOEC and NOEC determinations from the appropriate parameters (endpoints) were done using the ANOVA Procedure ( $\alpha = 0.05$ , one sided) and properly selected multiple t-tests using a commercial program. In case of a limit test (comparison of control and one treatment group only) the STUDENT t-test was used.

Calculations were carried out using Microsoft Excel® spreadsheets. All further statistical evaluations were done using the commercial program ToxRat Professional.

Chemical analysis of the pore water over time yielded 1.29% of nominal of day 0, 6.66% on day 7 and 7.29% on day 28 Biological results are reported as initial pominal concentrations. Detailed analytical results are presented in the following table:

Nominal and measured concentrations of 6 Chloropscotinic acid (AE F161089) Table:

& & & & & & & & & & & & & & & & & & &	c acid mg p.m./	L]	
Day 0 (1 hour) Y O O D		Day	<b>28</b>
West A Contraction of the Contra	₩ % of		% of
andytical nominal andytical	nominal	analytical	nominal
O O O O O O O O	ng water		
< LOQ		< LOQ	
, 1 <b>6</b> 2 , 162 , 89.6	89.6	90.6	90.6
pore water 1)			
LOQ		< LOQ	
22.9 12.9 103	6.66	91.9	7.29
	Day 0 (1 hour) Day 0	Day 0 (1 hour)	Day 0 (1 hour) Day 7 Day  analytical nominal analytical nominal analytical overlying water  - LOQ

<sup>1)</sup> Calculated to the real volume of pore water per lest vessel

# C. Biological Findings

Start of emergence was at day 13 for the controls and the limit test concentration of 100 mg p.m./L. 87.5% of the inserted (n= 120) larvae maturated to adults in the controls after 28 days. Observations on emergence sex ratio and development rate are listed as follows:

Table: Effect of 6-chloronicotinic acid on survival and development of Chironomus riparius

Nominal	Number of	Emergence of introduced larvae		Dovelonment rate	
concentration (mg p.m./L)	emerged midges (introduced)	total %	male %	female %	Development rate (1/d); pooled see
Control	105 (120)	87.5	41.7	45.8	≥ 0.064 D
100	106 (120)	88.3	50.0	38.3	0.066

The Chi<sup>2</sup>-Test indicates no statistically different distribution between sexes compared to the assumption of 50% females and 50% males. Therefore male and female results were pooled for further statistical analyses to increase the statistical power.

There was no statistical significant difference in emergence between the control and solvent control and at the limit test concentrations of 100 mg p.m./L as compared to the control finding. For the development rate (pooled sex) there was no statistical eignificant difference as compared to the control for the limit test concentration.

# D. Validity Criteria

Mean number of live offspring per parent was greater than 60 in the control. The emergence in the control(s) was > 70% of introduced larvae at the end of the test. The emergence was between 12 and 23 days after their introduction into the control vessels. The oxygen content in the water body was > 60% of saturation at the end of the test in all test vessels. The pH of the overtying water was between 6 and 9 in all test vessels. The water temperature aid not to differ by more than  $\pm 1\%$  over the whole exposure period.

# E. Biological Endpourts Derived

From the results presented above the following biological empoints based on nominal initial concentration, can be derived.

Endpoints (mg p.m./L)	, Q	76 // 19	a. Store
emergence rate (pooled sex)		≥ 100,	> 100
development rate (poole@sex).	Ŏ, ×	<u></u>	> 100

### CONCLUSION

The chronic effect of 6 Chloronicotroic acid (AE-F)61089 on growth and reproduction of *Chironomus riparius* can be quantified as an overall-NOEC of  $\geq$  100 mg pure metabolite/L, the limit concentration tested.

# IIA 8.3.2.3 Chronic toxicity for ropr. species of aquatic gastropod molluscs

This study is not riggered as By 1 02960 is not intended to be used directly on surface water (overspray). Acute to ricity to the saltwater mollusc *Crassostrea virginica*, indicates no particular concern to this taxonomic group Annex II, 8.11.) and therefore, chronic tests were not deemed necessary.

# IIA 8.3.3 Aquatic Field testing

The available studies on laboratory indicator species give a detailed picture of BYI 02960's toxicity to aquatic non-target organisms with a special focus on invertebrates. The derived endpoints provide a good overview on potentially sensitive species and fields of concern. A risk assessment for the intended

uses of BYI 02960 used as a plant protection product can be undertaken without the requirement for testing at (model) field scale. Therefore no additional semifield or field tests have been undertaken.

### **IIA 8.4** Effects on algal growth and growth rate (2 species)

Testing on algae was performed for BYI 02960 and several major metabolites, testing on the BY 02960-azabicyclosuccinamide was not performed as the indication from testing of the other metabolites did not indicate a concern. This is in agreement with the recommendation of the Guidance Document on Aquatic ecotoxicology which proposed a factor of 100 to the most sepsitive (parent) species to indicate a necessity for further testing. The difference for BYI 02960 between the toward to algae and chironomids is > 1000 allowing a significant margin of safety if the metabolite should be more to significant margin of safety if the metabolite should be more to significant margin of safety if the metabolite should be more to safety if the safety if the metabolite should be more to safety if the safet than the parent to algae.

Growth tests with algae are considered a chronic test as several generations and develop during the lest period of 72h.

Report:	KIIA 8.4/01; C.V. (2010) Toxicity of BYI 92960 Technical to the Green Alga Pseudokirchneriella subcapitata
Title:	Toxicity of BYI 92960 Technical to the Green Alga Pseudokirchneriella
Report No:	EBRVP030
Document No:	M-397552Q1-1
Guidelines:	EPA OPPTS 859.54000
	OEGE Guideinie 2014
	FIFRA 123-2 0
Deviations:	None O S S S S S S
GLP:	Yes (certified laboratory)

## EXECUTIVE SUMMARX

The aim of the study was to determine the effects of BYI 02960 (Sample description: TOX 08508-00 (Batch ID: BCCR74729-01-01); purity 96.2%) on the growth of green alga (Pseudokirchneriella subcapitata) 🖗

Cultures of Pseudokirchweriello subcapitata with an initial cell density of 10000 cells/mL were exposed in a static system over a period of 96 hour To nominal concentrations of 5.0, 10, 20, 40 and 80 mg a.i./L (corresponding to analytically verified concentrations of 5.9,11, 23, 47 and 95 mg a.i./L). In addition, a water control and solvent control were tested

72 and 96 hour growth rates based on cellulensitionand visual assessment of potential cell deformations were used as endpoints. No physical abnormativies were observed in the control and in the treatment The 96-hour-E<sub>r</sub>C<sub>8</sub> was >80 mg/s.i./L. the 96-hour-NOEC was determined to be 80 mg/s.i./L the highest concentration to the sted

### MATERIAL AND METHODS

### A. Materials

1. Test material

Test item: BYI 02960 Type of test material: Substance, technical Chemical state and description: Beige powder Batch number: 2009-000239 Sample description: TOX 08508-00

CAS name: 2(5H)-Furanone, 4-[[(6 chloro-3-pyridinyl)methyl

difluoroethyl)amino]

CAS#: 951659-40-8

ingligation of the state of the IUPAC name: 4-[(6-chloropyridin-3-ylmethy

2(5H)-one

Purity: 96.2% w/w

Storage conditions: Room temperatur

Water solubility:

# 2. Test solutions

Vehicle:

Concentration of vehicle: Method of preparation:

Soncoated for one our Water control and sol Controls:

Evidence of undissolved material,

### 3. Test organisms

Species: Common name:

Source: , USA, received

Maintenance f pre-culture

Temperature: Photograpiod:

# B. Study design and metho

2. Design of biological test

2. Design of biological test Subcapitate were exposed to BYI 02960 (purity 96.2%) in a static system over a period of 96 hours. This study was conducted up to the functional limit of solubility in the test system, which was determined to be approximately 80 mg a.i./L. Nominal concentrations were 5.0, 10, 20, 40 and 80 mg a.i./Ly In adottion, a water control and solvent control was tested. Each vessel (Sterile Erlengever Hasks covered by inverted glass beakers; 250 mL) filled with 100 mL filter sterilized 1xAAP media ( $\Omega$ 20 mm filter) with an initial pH of 7.5 ± 0.1, served as one replicate. At test initiation the cell density was 19000 cells/mL. The test was conducted with 3 replicates per treatment

### 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at 0 and 96 hours from all concentrations. High-performance liquid chromatography (HPLC) was used as analytical method. The limit of quantification (LOQ) was 0.62 mg/L. The range of linearity was 0.001 to 0.5 mg/L.

### 4. Observation and measurements

Growth rates, observation on cell abnormalities and physical-chemical water parameters were assessed as indicated below in the result section.

### 5. Statistical analysis

Raw or transformed data from treatment groups were compared to controls for formality and homogeneity of variance using the Shapiro-Wilks test and Levene's test of equal variance, respectively. If normality and homogeneity of variance were demonstrated for the raw of transformed values, parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test If normality and/or homogeneity of variance were not demonstrated of raw or transformed value nonparametric procedures were used.

The ranks of the raw values were determined, and then an analysis of variance and a one-tailed Dunnett's test were performed on these ranks. The EC 3, and the respective 5% confidence intervals, were calculated with help of regression analysis for cell density

### RESULTS AND DISCUSSION

# A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test so

Test temperature:

7.3 to 9.4 at 15st start and 10.0 to 10.2 at test fermination pH:

Photoperiod:

Light source white fluor scent

Light intensity: (Mean: 4036 lux (range: 3740 A540 kx)

Conductivity:

### B. Analytical Findings

Analytical verification of test solutions revealed measured concentrations of 5.9,11, 23, 47 and 95 mg a.i./L calculated as arithmetic mean. Biological results are reported as nominal. Detailed analytical results are presented in the following table.

Nominal and measured concentrations of BYI 02960 Table:

Nominal	Q measure	ed da 🕉 🦠	measur	d day 4	Mean	Mean %
Conc. (mg a.i.49)	≽mg a.i.∭	% nominal	mg a.i./L	% nominal	Measured	Nominal
Control	< L269Q ~	~ <sub>1</sub> Q′	\$ < LQ <b>Q</b> *		< LOQ	
Solvent Control	≨LOQ ₷		√ < <b>k</b> ØQ		< LOQ	
5	~5.8 °	117	<b>3</b> .9	118	5.9	117
10 🔬	114	1110	12	117	11	114
20		y 11%,	23	116	23	115
40	48	<u>_</u> <b>2</b> 20 🖧	46	115	47	117
80	<u></u> \$ 94 & €	√9118 °V	96	120	95	119

# C. Biological Findings

Observations of growth rates are listed as follows:

Table: Effect of BYI 02960 on growth-inhibition of Pseudokirchneriella subcapitata

Nominal	72-h-Mean	72-h-% inhibition	96-h-Mean	96-h-% inhibition
Concentration (mg a.i./L)	growth rate (cell density)	compared to pooled control	growth rate (cell density)	compared to pooled control
Control	0.069946	NA	0.059591	NA O
Solvent Control	0.067933	NA	0.059691	NA Z
Pooled Controls	0.06894	NA	0.059641	NA S
5	0.070266	-1.9	0.059024	
10	0.070646	-2.5 <sub>Č</sub>	0.05894	1.2 ×
20	0.071208	-3.3	0.060	~0.9~~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
40	0.070429	-2.2	0.0\$92	
80	0.069627	-1.0	0 <b>\$</b> \$9782	-8×2 por a
1.0				
The validity criteria were fulfilled.				
	l Growth Validity Crit		4 0 V	
	rease (minimum reco	amended multiplication	A factor of app	noximatery 144 (mean
factor is 16): of control and solvent control groups)				
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3) in the controls (exiterion is \$\frac{1}{2}\) 35% \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \				
Coefficient of variation for average specific growth rates during the 0 0 1.0% to 72 hour test period in replicate control cultures (criterion is 7%)				

### D. Validity Criteria

0 to 72 Hour Control Growth Validity Criteria:
Control Biomass Increase (minimum recommended multiplication
factor is 16):
Mean coefficient of variation for section-by-section specific growth
rates (days 0-1, 1-2, 2-3) in the controls (craterion in 35 35%)
Coefficient of variation for average specific growth rates during the 0
to 72 hour test period in replicate control cultures (criterion is 7%)

# E. Biological Endpoints Derived

From the results presented above the following biological

72 h and 96-h-figures (growth rate)

 $E_rC_{50}$ 

highest concentration with no effect (NOEC): Lowest concentration with effect (LOGC):

The effect of BYI 02960 on Pseudokirchireriell@subcapitata can be quantified as a 96-hour-ErC50 of >80 mg a.i./L. The highest concentration with no observed frowth inhibition and no cell deformations can be set to 80 mg a C/L, the highest tested concentration.

Report:	KIJA 8.4/02; Q2011)
Title."	Pseudokirchnericha subcapitata growth inhibition test with BCS-AB60481 – limit test
Report No:	NEBROYP077√C Q
Document No	M&09118-01-2 @
Guidelines	PECD Suideline 201
Deviations: Q	NoneO
GLP:	Yes (certified laboratory)

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the effects of sodium difluoroacetate (Na-salt of DFA; Sample description: TOX 08988-01; code: BCS-AB60481; purity > 99.0% w/w) on growth rate of Pseudokirchneriella subcapitata.

Cultures of *Pseudokirchneriella subcapitata* with an initial cell density of 10,000 cells/mL and 6 replicates were exposed in a static system over a period of 72 hours to a nominal concentration 10 mg p.m./L. In addition a water control was tested.

Quantitative amounts of BCS-AB60481 were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period. Analytical findings on day 0 and on day 3 were 103% of \$\infty\$ nominal and 100% of nominal, respectively. Based on analytical firelings, the biological endpoints are reported as nominal figures.

72 hour growth rate based on cell density was used to determine the endpoints. The 2-hour-E<sub>r</sub>C<sub>50</sub> > 10 mg/L, the 72-hour-NOFC was determined to by 10 mg/L. > 10 mg/L, the 72-hour-NOEC was determined t

### **MATERIAL AND METHODS**

### A. Materials

### 1. Test material

Test item:

Type of test material: Chemical state and description: & White powder !

Batch number:

Code:

Sample description: Purity:

Storage conditions

Vehicle: Control

Evidence of undissolved material

# 3. Test organisms

Psepolokirchaeriellosubcapitata Species:

Common mame: Collection of Algal Cultures, Source S

Maintenance of pre-culture: Permanent light Photoperio d

# B. Study design and method

December 3, 2010 to February 27, 2011

### 2. Design@f biological test

Green alga (*Pseudokirchneriella subcapitata*) were exposed to sodium difluoroacetate (Na-salt of DFA; code: BCS-AB60481; purity > 99.0% w/w) in a static system over a period of 72 hours. Nominal

concentrations were 10 mg/L. In addition a water control was tested. Each vessel (Erlenmeyer flasks; 300 mL) served as one replicate filled with 150 mL test solution. At test initiation the cell density was 10,000 cells/mL. The test was conducted with 6 replicates per treatment level.

# 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at Q and 72 hours. concentrations. HPLC-MS/MS was used as analytical method. The limit of quantification  $0.998 \mu g/L$ . The range of linearity was  $0.11 \mu g/L$  to  $11 \mu g/L$ .

### 4. Observation and measurements

Growth rates, observation on cell abnormalities and physical-chemical water parameters were assessed as indicated below in the result section. Growth inhibition was calculated using algae blomass per volume. The surrogate for biomass was cell density (used as response parameter), measurable by direct counting of algae cells per volume or indirectly by calculation of cell numbers after measurement of optical cell density.

### 5. Statistical analysis

Not applicable.

### RESULTS AND DISCUSSION

# A. Physical and Chemical Parameters

A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test solutions are summarized as follows:

Mean 21.4% (rango 21.3% to 22.0°C) Test temperature:

Ranged from 7.7 to 7.9 in the controls; ranged from 7.7 to 7.8 at 10 mg/L pH:

Permanont light Photoperiod:

Cool white fluorescent lamps (Sylvania Standard ICW / 133-T8) Light source

Light intensity: Mean 7280Mux (r@rge 7031) to

# B. Analytical Findings

Analytical verification of test solutions revealed measured concentrations calculated as arithmetic mean. Detailed analytical results are presented in the following table:

Nominal and measured concentration of BC AB60481 Table:

Nominal concentration [mg/L]	Concentration [mg/R] % necovery concentration [mg/R] % average day (b)	Measured concentration [mg/L] average day 3	% recovery
control 🗬	<0.000928	< 0.000998	-
10 mg/L	10,30′ 0′ 2103	10.0	100

# C. Biological Findings

Observations on growth rates are listed as follows:

Effect of BCS-AB60481 on 72-hour-growth-inhibition of Pseudokirchneriella subcapitata

	Cell Number/mL after 72 h (means)	(0-72h)-Average Specific Growth Rates [days <sup>-1</sup> ]	Inhibition of Average Specific Growth Rate
Contro	679 000	1.406	-
10	573 000	1.349	4

test initiation with 10,000 cells/mL

No cell abnormalities were observed.

### D. Validity Criteria

The validity criteria were fulfilled. Biomass increased in the control by more than 16-fold within the evaluation period. Mean percent coefficient of variation of sectional growth rates from day 0-1, day evaluation period. Mean percent coefficient of variation of sectional growth rates from day 0-1, day 1-2, and day 2-3 in the control did not exceed 35%. Percent coefficient of variation of the average growth rate in each control replicate did not exceed 7%.

E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived 72-hour-figures (growth rate):

ErCso
highest concentration with no effect (NOE, C):

10 mg p.m/L
10 mg p.m/L
10 mg p.m/L
10 mg p.m/L

The effect of sodium difluoroacetate (NQ-salt of BYI 02960 DFA Pseudokirchneriella subcapitata cambe quantified as a 72-hour DC50 of 10 mg p.m. The highest concentration with no observed growth inhibition and no cell deformations can be set to 10 mg p.m./L the limit concentration tested.

Report:	KILA 8.4/03; (2014) (2014)
Title:	Posudokirchneriella subgapitata growth inhibition test with BYI 02960 –
Report No:	succinating — agint test
Report No:	DEBRYP184 V V V V V
Document No:	M-474090601-2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Guidelines:	OECD Gaideline 201
Deviations:	None Q A A A A A A A A A A A A A A A A A A
GLP:	Yes (certified laboratory)

### EXECUTIVE SUMMARY

The objective of this 72 four growth inhibition test was, to verify the assumption that the BYI 02960 – succinamide (Sample description: TOX 093/43-00 (Batch ID: BCS-CR74729-01-01); purity 97.8% w/w) will cause no advers beffect on the growth of the green algae Pseudokirchneriella subcapitata.

Cultures of Pseudokirchnertella subcapitalla with an initial cell density of 10,000 cells/mL and 6 replicates were exposed in static system over a period of 72 hours to a nominal concentration of 10 mg p.m./L (limit test). In addition water control and a solvent control were tested.

Quantitative mounts of BX 02960 – succinamide were measured in all treatment groups and in the control on day 0 and day of the exposure period. The analytical findings of BYI 02960 – succinamide found on day 0 were 144 % of nominal. On day 3 analytical findings of 113 % of nominal were found. All results are based on nominal test concentrations of the metabolite.

72 hour growth rate based on cell density were used to determine the endpoints. The 72-hour-E<sub>r</sub>C<sub>50</sub> was > 10.0 mg pure metabolite/L, the 72-hour-NOE<sub>r</sub>C was determined to be 10.0 mg pure metabolite/L.

### MATERIAL AND METHODS

### A. Materials

1. Test material

Test item: BYI 02960 - succinamide

Type of test material: Substance, technical (pure metabolite)

Chemical state and description: White powder BCOO 6329-2-10 Batch number: BCS-CR74729 Code: Sample description: TOX 09343-00

CAS name: 4-{[(6-chloropyridin-3] yl)methyl]

oxobutyric acid

Purity: 97.8% w/w Storage conditions:  $+10 - +30^{\circ}$ 

2. Test solutions

Vehicle: Concentration of vehicle:

Controls:

Evidence of undissolved material:

3. Test organisms

Pseudokirchneriella subcapitata
Greenalga Species:

Common name:

Collection of Algal Cultures, Source:

Maintenance of pre

Temperature: Photoperiod:

B Study design and methods

1. In life dates

### 2. Design of biological test

Green algae (Pseudokir Chericifia sub Capitaia) were exposed to BYI 02960 – succinamide (purity 97.8% w/w) in static system over a period of 72 hours. Nominal concentrations were 10 mg p.m./L. In addition a water and a solvent control were tested. Each replicate consisted of a vessel (Erlenmeyer flasks; 300 mL) filled with 150 mL of test solution. At test initiation the cell density was 10,000 cells/mL. The test was conducted with 6 replicates per treatment level.

# 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at day 0 and day 3. HPLC-UV was used as analytical method. The limit of quantification (LOQ) was 136 μg/L. The range of linearity was 10 µg/L to 2186 µg/L.

# 4. Observation and measurements

Growth rates, observation on cell abnormalities and physical-chemical water parameters were assessed as indicated below in the result section.

### **RESULTS AND DISCUSSION**

### A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test solutions are summarized as follows.

Test temperature: mean 21.5°C (range 21.3°C to 22.2°C) pH: ranged from 7.8 to 8.2 in the controls

Photoperiod: permanent light

Light source cool white fluorescent lamps

Light intensity: mean 8417 lux (range \$220 to 8790 lux

# **B.** Analytical Findings

Analytical verification of test solutions determined in the concentration of BYI © 960 Succinamide on day 0 to be 114% of nominal and on day 3 to be 113% of nominal was found respectively. Detailed analytical results are presented in the following table:

Table: Nominal and measured concentrations of BY 02960 succinamide

Nominal concentration [mg p.m /L]	1. determination 2. determination werage %cecovers [mg p.m/L] kng p.m/L]
control	< 0.136 Q
solvent control	< 0.136 0.136 0.136 0.136 -
10 (day 0)	11.40 4 0 11.4
10 (day 3)	11.4 & 0 H.3 6 H.3 6 113

### C. Biological Findings ✓

Observations on growth rates are listed as follows:

Table: Effect of BYJ 02960 Succinamide on 72-hour-growth-inhibition of Pseudokirchneriella subcapitata

0		24 <b>dy</b> 0	& D D	48 h 🥎 🏻 🗳	Y	0 - 72 h	
Nominal 👸		Inhibition	« Average	Linhibition	«Cell	Average	Inhibition
Concen	Specifican	of a	Specific	of Ø	<b>Sumber</b> /	Specific	of
tration	Growth Rates	<b>Av</b> erage	Growth 6		mL after	Growth	Average
[mg p.m./L]	Rares		Rates	Specific		Rates	Specific
l gr j	[days <sup>1</sup> ]	Growth	days	Growth	(means)	[days <sup>-1</sup> ]	Growth
	à A	Rate [%] <		Rate [%]			Rate [%]
control	<u>.</u> 1.742 <sup>©</sup> 1	- Š - Š	Ĵ\$560 Å		753 000	1.440	-
solvent «	1.743		1 526		738 000	1.434	
control 4	1./13	<i>v</i> ≪() 4	1.536	Q1 -	738 000	1.434	-
pooled	1.727	Z Ó	12/10 %	<b>S</b>	746 000	1.437	
control	1./2/		\$340 N	_	740 000	1.43/	-
10.0	1,796	1.2	<b>®</b> 1.52 <b>5</b> √	1.5	743 000	1.435	0.1

No cell abnormalities were observed

### D. Validity Griteria,

The validity criteria were fulfilled

### E. Biological Endpoints Decived

From the results presented above the following biological endpoints can be derived:

 $E_rC_{50}$  > 10.0 mg p.m./L $NOE_rC$   $\geq 10.0 \text{ mg p.m./L}$ 

### **CONCLUSION**

The effect of BYI 02960 – succinamide on *Pseudokirchneriella subcapitata* can be quantified as a 72 hour- $E_rC_{50}$  of > 10.0 mg pure metabolite/L. The highest concentration with no observed growth inhibition and no cell deformations can be set to  $\geq$  10.0 mg pure metabolite/L the limit concentration tested.

Report:	KIIA 8.4/04; (2012)			
Title:	Pseudokirchneriella subcapitata growth inhibition	n test <i>©i</i> th 6-chl	loronicotinic ac	id "©"
Report No:	EBRVP242	R .		
Document No:	M-424145-01-2	A .	.O Q	,0° }
<b>Guidelines:</b>	OECD Guideline 201		4 4	
<b>Deviations:</b>	In the highest test concentration of 100 mg p.m	ı./L.6 Gest vesse	Is were used in	the 🔎
	study. In 3 of these vessels the pH was adjusted	lte∢8.2 (pHoofd	control) before	
	introducing the algae. The temperature ranged	Lbetween 19.4	and 23.0°C.	.4
	These deviations had no impact on the outcom	e of the study.		
GLP:	Yes (certified laboratory)	A	, ,	

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the influence of 6-chloropeotine acid specified by origin batch no.: M12653, certificate no.: AZ 16813 and LIMS no.: 022273, analysed purity: 98.8% w/w) on the growth rate of the green alga *Pseudokirchneriella subcapitata*.

Cultures of *Pseudokirchneriella subcapitata* with an initial cell density of 10 000 cells/mL were exposed in a static system over a period of 72 hours to nominal concentrations of 6.25, 12.5, 25.0, 50.0, 100 (pH not adjusted) and 100 pH adjusted) mg pute metabolite in comparison to a control.

In the highest test concentration of 000 mg p.m./L 6 test vessels were used in the study. In 3 of these vessels the pH was adjusted to 75 - 8,2 (pH of control before introducing the algae, whereas in the remaining (not pH adjusted) vessels the pH was 5 1 - 6.6

Quantitative amounts of 6 - chloroficotinic acid were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period. The analytical findings of 6 - chloronicotinic acid in the treatment levels found on day 0 were 100 % to 112 % of nominal (average 105 %). On day 3 analytical findings of 98.7 % to 100 % of nominal (average 105 %) were found. All results are based on nominal test concentrations of the metabolite.

The 72 hour growth rate based on cell deporty was used to determine the endpoints.

Test concentration of 100 mg p to L not pH adjusted (pH 5.1 − 6.6):

The  $E_rC_{50}$  was 114 mg p.m./L, the  $E_rC_{10}$  was 80.5 mg p.m./L and the NOErC was 50.0 mg p.m./L.

Test concentration of 100 pg p.pl./L pH adjusted (pH 7.5 – 8.2):

The  $E_rC_{50}$  was  $\gtrsim 400$  mg/p.m. (f), the  $E_rC_{10}$  was 130 mg p.m./L and the NOE<sub>r</sub>C was  $\geq$  100 mg p.m./L.

The observed results demonstrate that the effects detected at 100 mg pure metabolite/L are caused by the highly acidic nature of the solutions and are not of relevance in the natural environment, the proposed end points for risk assessment are therefore the value derived from the pH adjusted test.

The (0 - 75) -E  $_{r}C_{50}$  for 6 - chloronicotinic acid is  $\geq$  100 mg p.m./L,

the (0 - 72h) - NOE<sub>r</sub>C is  $\ge 100$  mg p.m./L.

### MATERIAL AND METHODS

### A. Materials

1. Test material

Test item: 6-chloronicotinic acid (6-CNA)

Type of formulation: Substance, technical (pure metabolite)

 $+5 \pm 5$ °C

Chemical state and description: Beige powder Batch No.: M12653

Code: AE F161089 00 1B99 (000)

Sample description: AZ 16813
Purity: > 98.8% w/w

2. Test solutions

Storage conditions:

Vehicle: None

Controls: Water control
Evidence of undissolved material: None

3. Test organisms

Species: Pseudokirofineriella subcoppitate

Source: Collection of Agal Coltures,

Maintenance of pre-culture:

Temperature: 19.4 to 23.0°C Photoperiod: permanent light

B. Study design and methods

1. In life dates November 11 to Desember 30, 201

### 2. Design of biological test

Green alga (*Pseudoterchneriella arbcapitata*) were exposed to the metabolite 6 - chloronicotinic acid in a chronic multigeneration test for 3 days under static exposure conditions to nominal concentrations of 6.25, 12.5, 25 0, 50.0,000 (pH not adjusted) and 100 (pH adjusted) mg pure metabolite/L. In addition, a water control was tested.

Each vessel (Erlenmeyer flasks, 300 mL) served as one replicate filled with 150 mL test solution. At test in that in the cell density was 10,000 fells/mL.

The test was conducted with 3 replicates per test level and 6 replicates per control.

# 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at 0 and 72 hours from all concentrations. We LC-MS/MS was used as analytical method. The limit of quantification (LOQ) was 0.11  $\mu$ g/L. The range of linearity was 0.11  $\mu$ g/L to 11  $\mu$ g/L, with an injection volume of 2.5  $\mu$ L. The correlation befficient was 0.9995.

### 4. Observation and measurements

Growth rates, observation on cell abnormalities and physical-chemical water parameters were assessed as indicated below in the result section. Growth inhibition was calculated using algae biomass per

volume. The surrogate for biomass was cell density (used as response parameter); measurable by direct counting of algae cells per volume or indirectly by calculation of cell numbers after measurement of Measurements of physical and chemical parameters of the test solutions are summarized as follows:

Test temperature:
pH:

Ranged from 7.8 to 8.2 in the controls from 7.8 to 8.6 in 6.2

8.1 in 12.5 mg/L; from 7.6 to 8.1 in 25 mg/k; from 3.1 to 6.2 in 100 mg/L (plknot adjusted)

Photoperiod:
Light source
Light intensity

Light intensity: Mean 793 Dlux (rapge 7430 to 853)

### **B.** Analytical Findings

B. Analytical Findings

Analytical verification of test solutions revealed measured concentrations calculated as arithmetic mean. Detailed analytical results are presented in the following table.

Nominal and measured concentrations of Schloronicotime acid Table:

		Al 37 . 7	· 0 - 1 6 4	
Nominal	Measured  concentration mg/L  average day/0		Measured 🐣	
concentration &	concentration mg/L	% recovery	concentration [org/L]	% recovery
[mg/L]	average day 0		concentration [org/L] average day 3	
control 🍣	<000009830° &		0.0009831	
6.25	6.9%	~112 ~	<b>6</b> 70	107
12.5		105	<b>2 2</b> 3.5	108
2590	\$.6	\ 1692 ×	© 26.3	105
50.0	\$\int \gamma \forall 50.4 \times 6 \times 6	√ <b>1</b> 701 ⟨⟨,	<b>△</b> 49.8	99.6
100	100		98.7	98.7
100 (pH adjusted)		1 1 1	110	110
	mean C	) <u>*</u> <b>19</b> 5	mean	105

No cell abnormalities were observed.

Observations on growth rates are listed as follows:

# Bayer CropScience

1 abic. Effect of 0 - chiof officolific actu off /2-ffout-21 owth-fillibition of 1 seadowi chief tend subcabillan	Table:	Effect of 6 - chloronicotinic acid on 72-hour-growth-inhibition of Pseudokirchneriella subcapitat	a
-------------------------------------------------------------------------------------------------------------------	--------	---------------------------------------------------------------------------------------------------	---

nominal concentration [mg p.m./L]	cell number after 72 h (means) per mL	(0-72h)-average specific growth rates [days <sup>-1</sup> ]	inhibition of average specific growth rate [%]
control	1 022 000	1.540	>
6.25	1 075 000	1.555	₹-1.0
12.5	1 277 000	1.612	<i>"</i> -4.7 ∼
25.0	1 543 000	1.679	-9.0
50.0	1 206 000	1.595	-3.6
100	238 000	1.055	31.50
100 (pH adjusted)	860 000	1.483	3,70

test initiation with 10000 cells/mL

# D. Validity Criteria

coefficient of variation of sectional growth rates from day 0-1, day 1-2, and day 2-3 in the control of not exceed 35%. Percent coefficient of variation of the average growth rate in each control replicate did not exceed 7%. The validity criteria are therefore fulfilled. Biomass increased in the control by more than 16-fold within the valuation period. Mean percent

# E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

Test concentration of 100 mg p.m./ not pl adjusted (pHS

Average growth rate  $(0 - \sqrt{2}h)$ 

Test concentration of 100

Average growth rate (0

## **CONCLUSION**

Test concentration of 100 mg p.m.

The (0 - 72h)-E<sub>r</sub>C<sub>50</sub> for e - chloronicotinic acid

the (0 - 72h)-NQ®₁Č

Test concentration of 100 mg p.m./L pH adjusted (pH 7.5 – 8.2):

The (0.72h)-6.50 for 6 - chiloronicotinic acid is > 100 mg p.m./L,

the  $(2)^{2}$  72h)  $E_{r}C_{10}$  is 130 and p.m./L,

the  $(0 - \sqrt[3]{2})$  - NOE<sub>r</sub>C is  $\geq 100$  mg p.m./L.

<sup>-%</sup> inhibition: increase in growth relative to the control

The observed results demonstrate that the effects detected at 100 mg pure metabolite/L are caused by the highly acidic nature of the solutions and are not of relevance in the natural environment, the proposed end-points for risk assessment are therefore the value derived from the pH adjusted test

The (0 - 72h)-E<sub>r</sub>C<sub>50</sub> for 6 - chloronicotinic acid is > 100 mg p.m./L,

the (0 - 72h) - NOE<sub>r</sub>C is  $\ge 100$  mg p.m./L.

### **IIA 8.5** Effects on sediment dwelling organisms

Based on the European triggers and the EU-Aquatic Guidence Document studies with sediment dwelling organisms are required if, in the water-sediment study, > 10% of applied radioactivity represented by the parent compound is present in the ediment at orgatter day 14, and the chronic No for Daphnia is < 0.1 mg/L. A further trigger may be a high acute toxicity to Chironograps in & combination with an occurrence in the sediment at greater than 10 %

In laboratory studies (see Section 5, point 9.7) BYI 02960 was found in segment in amounts exceeding 10%, however under more natural conditions, but still confined conditions of an outdoor micro-cost study the amount of BYI 02960 was must lower (< 10% for the test of 10 µg/L).

The test species recommended for testing sediment dwelling organisms is Chiramomus Eparius, this species has been tested as a second sensitive indicator species for invertebrates. For BYI 02960, the acute toxicity to Chironomus was considerably drigher than that observed Daphnia Considering the physico-chemical properties of BYI 02960 (Quatic solubility and log Koo) a spiked water test is considered more relevant for the use of BXY 02960 to determine the risk to benthic organisms. This test was summarized under 8.2.3.3 above but is considered to address also the risk to sediment dwelling organisms.

For the metabolites BYI 02960 Overe is fittle concern for the centhic compartment as the  $K_{OC}$  values are very low, testing on chironorbids indicates that the metabolites we much less toxic than the parent and further testing is not required.

According to US and Canadian directives toxicity studies with sediment organisms are not required due to the low Coc-value of BYI 02960 and the metabolites suggesting no relevant exposure of the sediment compartment

IIA 8.5.1 Acute test

An acute test to the test species Chironomus ripaijus was summarized under 8.3.1.2. This test allows conclusions on the intrinsic toxically to the midge. However, a sediment substrate is not included into the test. For reasons of completeness, the summary of the conclusions of the test is summarized here again 👟

Report:	KIIA, 8:5.1/01; (2011)
Title:	Acute toxicity of BYT 2960 (tech.) to larvae of <i>Chironomus riparius</i> in a 48 h static
	laboratory test system
Report No.	₽BRVPQ26 ~~
Documen No: 0	M-41 39-01-2
Guidelines:	No pecified guideline; study is performed according to general aspects as
	and the control of th
Deviations:	According to test system
GLP:	Yes (certified laboratory)
	Screening of water for contaminants was not performed under GLP as
	described in the study report

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the acute effects of BYI 02960 (Sample description: TOX 08508-00 (Batch ID: 2009-000239); purity 96.2%) on larvae of *Chironomus riparius*.

Following 48 h of exposure 0, 0, 2.5, 2.5, 30, and 85% immobilisation was observed among dappenids exposed to the 3.125, 6.25, 125.5, 25.0, 50.0, and 100 mg a.i./L treatment levels respectively. No immobilisation occurred in the water control. Therefore, the 48-hour-EC<sub>50</sub> was 61.7  $\mu$ g a.i./L (95%) confidence limits 41.4 to 109  $\mu$ g a.i./L), the 48-hour-NOEC was determined to be 25  $\mu$ g a.i./L.

### IIA 8.5.2 Chronic test

The testing rationale was described under section \$5., chronic spiked water test with the recommended species, *Chironomus riparius*, are available for BYI 02%0, DFA and 6 CNA and sommarized under 8.3.2.2. For reasons of completeness, the conclusions of test for the parent compound are summarized, here again.

Report:	KIIA 8.5.2/01
Title:	Chironomio riparjus 28-day chronic toxicity test with BY 192960 (tech.) in a water-
	Chironomic riparjus 28-day chronic toxicity test with BY 02960 (tech.) in a water-sediment system using spiked water
Report No:	EBRY P025
Document No:	M-401792-61/-2 Q
<b>Guidelines:</b>	QECD Guideling 219 &
Deviations:	None A S S S S S S S S S S S S S S S S S S
GLP:	Yes (certification and the second sec
Q'	Screening of contaminants in water was not performed according to GLP

### EXECUTIVE SUMMARY

The aim of the study was to determine the effects of BYF 02960 (Sample description: TOX 08508-00 (Batch ID: 2009-000239) purity 96.2% w/w) on emergence and development of *Chironomus riparius*.

The start of emergence was reduced for one day at test concentration of 20.0 μg a.i./L. No emergence was observed at the highest test concentration of 40.0 μg a.i./L. The NOEC based on emergence rate and development rate was determined to be 10.0 μg a.i./L. (pritial measured: 10.5 μg a.i./L..

# IIA 8.6 🐧 Effects of aquatic plants 👌

In Europe sests on aquatic plants are not required for non-herbicidal substances. Hence, as BYI 02960 is a selective insecticide tests on higher plants are not required.

For The US and Canada data must be provided for the macrophyte *Lemna*, therefore a 7 day growth inhibition test is available. Agrhere is no indication that the metabolites have a relevant activity no testing for the metabolites has been performed.

A general comment is considered appropriate with respect to the evaluation of effects in *Lemna* studies based on two parameters, i.e. frond number and dry weight or total frond area. "Growth rate" is the only and relevant response variable, which can then be used in risk assessments. This issue is addressed within the DECD guideline 221 in paragraph 51 (finalized in 2006). It is clearly stated that the response variable average specific growth rate" is independent of the absolute level of the specific growth rate of the control, slope of the concentration-response curve or on test duration, and is therefore the preferred measure for reliable endpoint calculations. The response variable "yield" is a USEPA national

requirement and, although calculated and reported, should not be used for comparing the toxicity of toxicants.

toxicants.	O.
This argumentation in more relevant than the 2002).	OECD 221 reflects the actual status of scientific discussions, and is considered corresponding chapter 2.4.1 in the Aquatic Guidance Document (issued in
Report:	KIIA 8.6/01; , C.S., J.M & C.V. (2010) Toxicity of BYI 02960 Technical to Duckweed (Lemna gibba CV) Undo Static
Title:	Toxicity of BYI 02960 Technical to Duckweed (Pemna gibba CS) Undo Static Renewal Conditions
Report No:	EBRVP043
Document No:	M-398376-01-1
Guidelines:	OECD Test Guideline 221: FIFRA Guideline 123-2 OPPTS 850.4400
<b>Deviations:</b>	None & Y Y Y Y Y Y
GLP:	Yes (certified Laboratory)

EXECUTIVE SUMMARY

The aim of the study was to determine the effects of BY I 02960 (Sample description: TOX 08508-00) (Batch ID: 2009-000239; purity 96.2%) on growth of duck weed (Leming gibba).

Cultures of Lemna gibba with an initial density of 12 fronds per vessel were exposed in a static renewal (one renewal at day 3) system over a period of days to normal concentrations of 5.0, 10, 20, 40 and Land Control (< 0.5 mg a.i and And frond dry weights from the control of the cont 80 mg a.i./L (corresponding to analytically verifical concentrations of 402, 8.19, 16.0, 34.2 and

Test item: BYI 02960

Type of test material: Substance, technical Chemical state and description: Beige powder Batch number: 2009-000239 Sample description: TOX 08508-00

CAS name: 2(5H)-Furanone, 4-[[(6-chloro-3-pyridinyl)methyl],2,2-

difluoroethyl)amino]

CAS#: 951659-40-8

4-[(6-chloropyridin-3-ylmethyl)(2,2-difluorgethyl)amino]fu@n-IUPAC name:

96.2% Purity:

Room temperature Storage conditions:

Water solubility: Approximately 80 mg a.i./L under te

2. Test solutions

Vehicle: Dimethylfornamide

Concentration of vehicle: 0.1 mL/L

Highest test level conicated approximately 4 hours (Daya) and approximately 5 Nours Day 3 Sinverted several times. Remaining test levels inverted several times. Water control and solvent control Method of preparation:

Controls:

Evidence of undissolved material:

3. Test organisms

Lemna Abl Duck meed Species: Common name:

Strain: Source:

Maintenance of pre-

Temperature: Photoperiod:

B. Study design and meth

Duck weed (Lenna gilla) were exposed to BYI 02960 (purity 96.2%) in a static renewal (one renewal at day 3) system over a period of Trays. Noming concentrations were 5.0, 10, 20, 40, and 80 mg a.i./L. In addition water control and a solven control were tested. Each vessel (borosilicate glass crystallisation dishes; 230 mL) filled with 100 mL 20xAAP with an initial pH of 7.5  $\pm$  0.1, served as one replicate. At test initiation the number of freeds was 12 fronds per vessel. The test was conducted with 3 replicates per treatment level.

3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at day 0 (new), day 3 (new and old) and day 7 (old) from all concentrations. BYI 02960 (99.4% w/w) served as analytical standard. The Finit of quantification (LOQ) was 0.5 mg a.i./L.

### Observation and measurements

Growth ates, observation on cell abnormalities and physical-chemical water parameters were assessed as indicated below in the result section.

### 5. Statistical analysis

Raw or transformed data from treatment groups were compared to controls for normality and homogeneity of variance using the Shapiro-Wilks test and Levene's test of equal variance, respectively. If normality and homogeneity of variance were demonstrated for the raw or transformed values, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test of the normality and/or homogeneity of variance were not demonstrated on raw or transformed values, nonparametric procedures were used. The ranks of the raw values were determined, and then an analysis of variance and a one-tailed Dunnett's test were performed on these ranks. Statistical analysis were conducted using SAS.

### RESULTS AND DISCUSSION

### A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test solutions are summarised as follows:

Photoperiod:

Light intensity: 5070 to 6370 lux (mean: 5764 lux)

Conductivity: 1500 to 1570 \text{ \text{µmh} \text{s}/cm} (r0 \text{can: 1553 \text{ \text{µmh} \text{cs/cm}})

### **B.** Analytical Findings

Analytical verification of test solutions resulted in measured concentrations of <0.5 control and solvent control), 4.02, 8.17, 16 % 34.2 and 67 mg a.P./L (80 to 86% of nominal) calculated as arithmetic mean. Based on these analytical findings the biological endpoints are reported as mean measured figures. Detailed analytical results are presented in the following table:

Table: Soming and measured concentrations of BYI 02960

	Day	60	Day 3	(old)	Day Day	(new)	<∪ Day	7	Me	an
Nominal	Mea-	%% nomi-	Mea-	% Snomi- «	measured Conc. (mg/a.i./L)	Day 3	₹ Mea-	Day 7	Mean	Mean
Conc.	🖓 sured	nomi-	spred	🔊 nomi- 🔻	Conc.	/ Miew) %	sured	%	mea-	mea-
(mg	Conc.	<b>≱</b> nal	Conc.	nælÇ	(mg/a.i./L)	nominal	Conc.	nomi-	sured	sured
a.i./L)	(mg 4	Q' /	≽ (mg⊗	°~			(mg	nal	Conc.	%
	a.i./L)		(mg a.i.W)	nad		Ö	a.i./L)		(mg	nomi-
		Q"		V//P		~~~			a.i/L)	nal
Control	< 0%	Q_'	O.5	0	\$ <b>0</b> .5		< 0.5		< 0.5	
Solvent	«Ő.5	~ %	~ . ~	,Q,"	Q < 0.50		< 0.5		< 0.5	
Control	<i>←</i>		* < <b>Q</b>		L // X //		٧٥.5		٧ 0.5	
5	3.39	~6 <b>%</b>	<b>Q</b> ,55	71	4.46	89	4.67	93	4.02	80
10.,	6.7	<b>≈</b> C67	<u>7.2</u>	727	<b>9</b> .14	91	9.63	96	8.17	82
10	13.1	66 S	7.2 7 144	72\$ Q1	<b>\$</b> 17.2	86	19.6	98	16	80
40	26 🐧	65	, <b>(9</b> )	a, 72 "	41.7	104	40.3	101	34.2	86
80	52 A	<sub>4</sub> 65	<b>⊘</b> 61.7 ≪	ž 77.Q	77	96	79.9	100	67.7	85
13.1   10.1   17.2   17.2   17.2   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1   10.1										



### C. Biological Findings

Mean measured test levels [mg a.i./L]	Final frond no. (replicate means, day 7)	Growth Rate for Frond Numbers [day-1]	% inhibition (growth rate for frond no.)
Control	238	0.017771	A
Solvent Control	217	0.01722	·
Pooled Controls	228	0.017496	W
4.02	208	0.016966	3.6
8.17	215	0.017145	260
16.0	191	0.016469	<b>*</b> 5.9 &
34.2	219	0.017226	× 1.5,0
67.7	169*	0.01&701* &°	3° 193° 40

<sup>\*</sup> Statistically different from controls (p<0.05)

C. Biological Findi	ngs				
Growth inhibition w	as observed as li	sted below.		2	
		wth-inhibition (frond nu	mbor) of Lamna aibb		
		`	midel) of Lemna gibb		
Mean measured	Final frond	Growth Rate	% inhibition		
test levels	no. (replicate	for Frond Numbers	(growth rate for	Ş	
[mg a.i./L]	means, day 7)	[day <sup>-1</sup> ]	frond no.)		
Control	238	0.017771		, O' , B' , A'	
Solvent Control	217	0.01722	<u> </u>		
Pooled Controls	228	0.017496	<u>V</u>		
4.02	208	0.016966	3.05		
8.17	215	0.017145	<b>2</b> (ø		
16.0	191	0.016469	<b>3</b> .9 66°		
34.2	219	0.0172	1.5		
67.7	169*	0.015701* 0.0000	5 193 5		
Mean measured	Dry weight	wth-inhibition (dry weig	ht) of Leming gibba  %inhibition (growth rate for dry weight)		
test levels	(means, day 7)		(growth rate for	¥ 5 . S	
[mg a.i./L]	[g] Q	[day-1]	ary weight)		
Control	0.0272	0.00 6273 C	\$\partial \partial \part	-8 <u>-</u> 4	
Solvent Control	0.0252	v 013011	X 0, 0		
Pooled Controls	0.0262 <u>%</u> 0.6238 O	0.016642		4 .0	
4.02 8.17	0.0249	0.015467	2.0	- 2	
	0.0249	0.01520 ×		- <b>o</b> j	
16.0 34.2	0.0230	0.01326	10	7	
	A. O			-	
67.7 \$\ \( \text{9} \)\( \text{251} \) \( \text{\$\sqrt{0.014814}} \) \( \text{\$\sqrt{0.7.7}} \) \( \text{\$\sqrt{0.7.7}} \)					
No cell abnormalities	es were observed.		-1.9 Y		
D. Validity Criteri	a & &	me less than 60 hours			
The validity criterio	n <b>Qua</b> doubling ti	me less than 60 hours	(2.5 days) in the con	trol is fulfilled.	
F. Biological Endn	Onte Dorivado				

# D. Validity Criteria

# E. Biological Endpoints Derived

biological endpoints can be derived: From the results presented above

Test Substance	BYI 02960 Technical
Test Object	Lemna gibba G3
Exposure 2 2 2	7-Day, static-renewal
7-day ErC 50 - growth rate for front numbers	> 67.7 mg a.i./L
7-day E <sub>b</sub> C <sub>50</sub> – cumulative biomass for frond numbers	> 67.7 mg a.i./L
7-day EC <sub>50</sub> – fregod count \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	> 67.7 mg a.i./L
7-day EC <sub>50</sub> — and dry weight	> 67.7 mg a.i./L
7-day E <sub>r</sub> C <sub>50</sub> growth rate for frond dry weight	> 67.7 mg a.i./L
Lowest Concentration With an Effect (LOEC)	67.7 mg a.i./L (frond counts)
Highest Concentration Without Toxic Effect (NOEC)	34.2 mg a.i./L (frond counts)

The NOEC and LOEC in the 7-day exposure study of *Lemna gibba* G3 to BYI 02960 technical were 34.2 and 67.7 mg a.i./L, respectively for the endpoints of 7 day frond counts, cumulative biomass for

fronds and growth rate for frond counts. EC50 values could not be calculated for any end-point and were determined to be greater than the highest test concentration (> 67.7 mg a.i./L).

### **IIA 8.7** Effects on bees

The acute oral and contact toxicity had been determined for BYI 02960 and plantmetabolites #8 bees could potentially be exposed, these include the metabolites BYI 02960-DEAF, BYI 02960-O BYI 02960-CHMP, DFA, 6-CNA.

Additionally 10 day feeding studies have been performed for BYI 02960 the metabolites BYI 02 DFEAF, BYI 02960-OH, BYI 02960-CHMP, DFA, 6-CNA, these studies are summarised under point

A study on the effects of exposure af larvae to BY \$2960 has also been performed and is summarised under point 8.16.

IIA 8.7.1 Acute oral toxicity

Report:	KIIA 8.7.1/802, & T. (2008)
Title:	Effects of BVI 02960 (Acute Contact and Oval) on Honey Bees (Apis mellitera L.) in
	the Laboratory & & & & & & & & & & & & & & & & & & &
Report No:	41121085
Document No:	M-308904 <sub>2</sub> 02-1
<b>Guidelines:</b>	OECD Guideline 213 O O O O O O O O O O O O O O O O O O O
	QECD Guideline 214 & A A A A A A A A A A A A A A A A A A
<b>Deviations:</b>	None None
GLP:	Yes@ertified laboratory)

The aim of the crudy was to determine the effects of BY102960 Sample description: TOX 08080-00 (Batch ID: NL 7780-44-6); purity. 99.5% w/v) on mortality of the honey bee (Apis mellifera) after oral or contact exposure

In the oral dose response test 30 adolt worker honey bees were exposed for 48 hours to doses of 2.8, 2.1, 1.3, 0.68, 0.34 and 0.17 µg all per bee by feeding (values based on the actual intake of the test item). For the contact dose response test 30 honey bees work exposed for 96 hours to doses of 200.0, 100.0, 50.0, 250 and 42.5 upa.i. per bee by topical application. The contact toxicity test was prolonged for 48 hours due to increasing mortality between 24 and 72 hours, up to a maximum of 96 hours.

In addition, negative controls foral: a) 50% aqueous sugar solution and b)acetone/sugar solution; contact test: a) tap water + 45% Adhäsit and b) acetone] and a toxic reference (Dimethoate; 400 g/L nominal) at nominal dose rates of 0.30 0.15, 0.08 and 0.05 µg dimethoate/bee were tested.

In the oral toxicity test, no mortality occurred in both the negative control and the solvent control group. The oral LDs values (24 + 8 h) of the test item BYI 02960 were calculated to be 1.3 and 1.2 µg a.i./bee, respectively. Behaviogral abnormalities (e.g. movement coordination problems and apathy) were of served on the four highest treatment groups during the first 4 hours in the oral toxicity test. After 24 hours and 48 hours no behavioural abnormalities were observed among the surviving bees.

In the confidence toxicity test, 10% and 3.3% mortality occurred in the negative control (water + 0.5%) Adhäsit) and the solvent control (acetone) group, respectively. The contact LD<sub>50</sub> values (24, 48, 72 and 96 h) of the test item BYI 02960 were determined to be >200, >200, 158.4 and 122.8 μg a.i./bee,

respectively. No significant test item related behavioural abnormalities occurred in the contact toxicity test except for a few bees among the two highest dose groups (200.0 and 100.0 µg a.i./bee) showing movement coordination problems and/or apathy from 48h onwards.

### MATERIAL AND METHODS

### A. Materials

1. Test Material

Test item: BYI 02960 Type: Substance, technical Chemical state and description: Solid, pink

NLL 7780-4 Batch No .: Sample description:

TOX 08080-00
99.5% www.according to certificate of analysis 2(5H)-Furanone 4-[[(e-hlorog-pyridinyl)methyl](2.2, Purity: CAS name:

difuoraethyl)aminol

951639-40-8 CAS#:

44/6-chloropyrid@-3-ylmeth IUPAC name:

¥5H)€one

Solubility:

In acetone not available acstart of tudy

Expiry d@e: 16,67.2008 when in @riginal Stability of test compound:

container in the dark

2. Vehicle and/or positive control 

O

Oral Test:

0% www sugar (syrus) solution (in tax wat at

(%5% sugar Flution with speed (%5% water, 5% acetone, 50%

Contact Test: a) Tap water with 0.5% Adhäsit (applied after anesthetization with

b) Accione (applied after an entertization with CO2)

(Adhäsit improves spreading of the test droplet on the waterrepellent hairs on the thorax of bees)

Wetting Agent:

Name: Batch No .:

Nominal content of active v @00 g/L Marlo@n (no@nal) Spies Urania Chemicals GmbH, Manufacturer:

Heidlenkarinsweg 7, 20097 Hamburg, Germany

Expiry date: 12/2009, when stored in original container, at room Stability:

temperature (19°C to 30 °C), in the dark

Target Amount in this Stud

Reference Item:

The information concerning the reference item according to the substance container label and data sheet:

Name: Perfekthion EC (BAS 152 11 I)

Manufacture BOSF AG, Agricultural Center Limburgerhof, D-67114

Limburgerhof

1814 Batch No

Nominal content of active ingredient: Dimethoate: 400 g/L

Analytical content of active ingredient: Dimethoate: 395.9 g/L according to certificate of analysis

Certificate of Analysis Study Code: 330040 1 Insecticide Chemical state and description: Liquid, blue 1.066 g/cm<sup>3</sup> Density:

Solubility: In water: emulsifiable

Stability: Expiry date: 01.11.2008, when stored in refrigerator  $(4 \pm 4^{\circ}C)$ , in

original container in the dark.

In water: reference item is considered stable under test conditions

### 3. Test organisms

Species:

Common name:

Age or developmental stage at test start:

Female adult worker bees
Disease-free and queen-right honeybee columes, bred by IBACON Source:

### B. Study design and methods

1. In life dates April 23 to July 13, 2008

### 2. Experimental treatments

Test units were stainless steel chambers of the cm x centilation holes (Ø 12 mm) side was a removable glass sheet, the bottom was perforated with 98 inner walls were lined with filter paper

(id. 30 jadividudis per treatment 10 bees were used per replicate unit replicates per group).

Exposure time for both tests was 48 hours. The contact test was prolonged for a further 48 hours due to increasing mortality between 24 and 72 hours up & a maximum of 96 hours

Food was commercial ready-to-rise syrup (Aprilivert: 60% Saccharose, 31% Glucose, 39% Fructose) ad libitum was supplied via syringes directly after treatment.

Bees in the oral test were starved for 20 minutes prior to test start.

Bees in the contact test were an esthetized for ca. 20 seconds with CO<sub>2</sub> until they were completely immobilized in mediately before application (only in the contactiest).. @

Control:

b) CO2/acetone

Aqueous sugar solution + acetone

Test item:

Contact Test

Nominal dosage 0 and 12.5 μg a.i./bee

Oral Tes

Nominal dosage 0.63, 0.31 and 0.16 μg a.i./bee

.3 0.68, 0.34 and 0.17 µg a.i./bee

Toxic reference item?

nina dosag 0.30, 0.20, 0.15 and 0.10 µg Dimethoate per bee

Oral Test

Nominal dosage 0.30, 0.15, 0.08 and 0.05 µg Dimethoate per bee

<sup>&</sup>lt;sup>1</sup> Adhäsit was used to improve the spreading of the test droplet on the bee body. Adhäsit is non-toxic to honey bees.

Measured dosage 0.26, 0.16, 0.08 and 0.06 μg Dimethoate per bee

### Application of the test item in the contact test:

Bees were anaesthetized with  $CO_2$  in the contact test. A single 5  $\mu$ L droplet of B 1 02960 in an appropriate carrier (acetone) was placed on the dorsal bee thorax using a Burkart – Applicator. For the control one 5  $\mu$ L droplet of a) tap water containing 0.5% Adhäsit\* and b) pure acetone was used. The reference item was also applied in 5  $\mu$ L (dimethoate made up in acetone). A 5  $\mu$ L droplet was chosen in deviation to the guideline recommendation of a 1  $\mu$ L droplet, since a higher volume ensured a more reliable dispersion of the test item; Ibacon experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected.

# Application of the test item in the oral test:

Appropriate amounts of BYI 02960 or reference item anutions in acctone were mixed with syrup (ready-to-use syrup; Apiinvert, Suedzucker, D-97195 Ochsonfurt; composition of the sogar component: 30% Saccharose, 31% Glucose, 39% Fructose) in order to achieve the required test concentrations on a final dilution of 50% syrup solution (45% water 50% syrup and 5% acetore (w/w)). For the controls, the same proportion of syrup, water and acetorie was used solven control and similarly, 50% aqueous syrup solution was used for the negative control. The treated food was offered in syringes, which were weighed before and after introduction into the cages (paration of uptake was 1 – Shours for the test item treatments). After a maximum of 6 hours, the syringes containing the treated food were removed, weighed and replaced by ones containing test, untreated food.

The target dose levels (e.g. 5.0 star a.i./bee nominal) would have been obtained if on mg/bee of the treated food was ingested. In fractice higher (or lower) dose levels were obtained as the bees had a higher or lower uptake of the test solution than the nominal 25 mg/bee.

### 3. Observation and measurements:

The number of dead bees was determined after 4 hours (first day); 24 and 48 hours (contact and oral test); 72 and 96 hours (contact test). Behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4 hours (first day); 24 and 48 hours (contact and oral test); 72 and 96 hours (contact test).

### 4. Statistical analysis

Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the control in both the confact and oral tests.

The contact and oral LD<sub>50</sub> of the test item were estimated with Probit Analysis (according to Finney 1971). The contact and oral LD<sub>50</sub> of the reference item were estimated according to moving average computations (Thompson and Weil, 1952). The LD<sub>50</sub> calculation was carried out taking into account the mortality data coffected by control mortality sing Abbott's formula (1925).

The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater,  $\alpha = 0.05$ ), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis.

The software used to perform the statistical analysis was ToxRat Professional, Version 2.09 and 2.10, ® ToxRat Solutions GmbH, © 2005.

### RESULTS AND DISCUSSION

### A. Environmental Parameters

Measurements of climatic parameters during the test are summarized as follows:

25°C Test temperature: 43 to 71% Relative air humidity:

Light intensity:

ventilation to avoid possible accumulation of pesticide vapour.

Test conditions were continuously recorded with an electronic data logger and documented in the raw data Ventilation to avoid possible accumulation of pesticide vapority. Test conditions were continuously recorded. Ventilation: Recording:

# **B.** Biological Findings

### **Contact Test:**

Mortality occurred in all treatment groups in a doso related pattern. The contact rest was protonged for a further 48 hours up to 96 hours due to increasing mortality between 24 and 72 hours. The contact dose levels of 200.0 to 12.5 µg a.i./bee resulted in mortality ranging from 73.3% to 6.7% at the end of the test (96 hours after application).

The mortality in the water control and the solvent control were 10.0% and 3.3%, respectively.

A prolongation was necessary because mortality was still increasing between 24 and 72 hours

Behavioural abnormalities (e.g. movement coordination problems) were found in a single bee at 50.0 μg a.i./bee dose level during the 4-hour assessment. These behavioural impairments were found at two highest dose levels from 48 to 96 hours following the application. &

There were behavioural abnormalities consistent with the observed toxicity in the reference item test.

Mortality and behavioural abnormalities of the bees to the contact toxicity test Table:

	T.	10		a. 🔊	′ <b></b>		a li			
	after 4 l	nours	after 2	hours 💜	after 48	<b>b</b> ours	after 72	2 hours	after 96	hours
	mortality	behav.	nnortality)	behav.	"mortality Q	behav.	mortality	behav.	mortality	behav.
	Ĉĩ,	\abnorm.\s\		ábnorm.	T S	abnorm.	19	abnorm.		abnorm.
dosage	Onean C	mean	pacan	a Omean 🎸	mean,	mean	mean	mean	mean	mean
[µg a.i./bee]	O % \$\frac{1}{2}\text{!}	269	. %	*\ %. Q^		O % . (	W %	%	%	%
test item 💍	0	_	, Q A		<i>®</i> ′ <i>a</i>	. 000	1			
200.0	0.0	×40.0	0.00	0.0	36.7	64	60.0	33.3	73.3	26.7
100.6	3.3	0.0	3.5	Q.0 6	23.34	6 <b>⊘</b> ) °	33.3	6.7	36.7	10.0
50.0	0.0	ر الإقراد	<b>€6</b> .7	\$ 0.0 \$	6.7	XX.0	20.0	0.0	23.3	0.0
25.0	0.0	es ea	$\sim 0.0$	0.0	6.4	$^{20}0.0$	13.3	0.0	13.3	0.0
12.5	9-90 <sup>-7</sup>	<u>1</u> 0.0	<b>♥</b> ゛0.0 <b>≪</b> √″	<b>Q</b> . <b>9</b>	V.0 8	0.0	0.0	0.0	6.7	0.0
water	00	0.0	0,60	°>Ø.0 &	<b>3</b> 0.0 <b>3</b>	0.0	6.7	0.0	10.0	0.0
solvent	Ø 0.0	0.0	~0,0	$\bigcirc 0.0 \bigcirc \bigcirc$	0.0	0.0	0.0	0.0	3.3	0.0
reference item	) O	%a√n	<b>\</b> ' \							
0.30	33.3	26.7	F 96.7	~Q.0	£J96.7	0.0	96.7	3.3	96.7	3.3
0.30	3.3		80.0	<b>20.0</b>	<b>90.0 90.0 90.0 90.0</b>	0.0	96.7	3.3	96.7	3.3
0.15	0.0	0,0	39.0	% 0.0 °	36.7	0.0	43.3	36.7	43.3	16.7
<u>√0°.</u> ¥0	0.0 <b>%</b>	.0×0	0.0	0.00	0.0	0.0	0.0	10.0	6.7	6.7

results are averages from three repulates (tembees each) per do sage/control

behav. abnorm. = behavioural abnormalities; water COwater treated control

Mortality occurred in all treatment groups (except at 0.34 µg a.i./bee): oral doses of 2.8, 2.1, 1.3 and 0.68 μg/θee, respectively, resulted in mortality ranging between 100.0 and 6.7% (48 hours after application). 3.3% mortality occurred at 0.17 µg/bee.

There was no mortality in the water and the solvent control.

During the 4 hours check, dis-coordinated movements and apathy occurred in the dose level of 2.8, 2.1, 1.3 and 0.68 µg a.i./bee groups, respectively. After 24 and 48 hours no further behavioural impairments were observed in any of test item treatment groups.

1.3 and 0.68 µg					hours n	o further b	ehavioural i	mpairments	
	were observed in any of test item treatment groups.  There were behavioural abnormalities consistent with the observed toxicity in the reference item sest.								
Table:	Table: Mortality and behavioural abnormalities of the bees in the oral toxicity test								
	after 4	hours	after 24	hours	after 4	18 hour <u>s</u> €	_		
	mortality	behav.	mortality		mortality	behav.			
uptaken	•	abnorm.	Ž	abnorm		norm.			
dosage	mean	mean	mean	mean, W	mean	R mean	, Ø .		
[µg a.i./bee]	%	%	%	% T	% &	%	_		
test item					Q,	,			
2.8	73.3	26.7	100.0	Ø.0	100,0	<b>©</b> 0.0 <i>°</i>	\$ \O'		
2.1	46.7	53.3	90.0	<sup>™</sup> 0.0 <sub>0</sub> ∘	<b>.99</b> .0	0.00			
1.3	46.7	40.0	53.3	′0.00°	€53.3 ×	00		4	
0.68	0.0	6.7	6.7 <sub>4</sub>	<b>6</b> .0	6.70	<b>©</b> 0.0		\$ 4°	
0.34	0.0	0.0	0.0	%0.0, %	0.0	<u></u> 0.0			
0.17	0.0	0.0	<b>6</b> 0 °	9 0.0	(S.3	% 0.20 \\	_ 🔊 🐇		
water	0.0	0.0	0.0 , 4	040	Y 0.0 X	0.0			
solvent	0.0	0.0	0.0	~ <b>_0.0</b>	0,00			, Ö	
reference item		Ŕ		) D				*	
0.26	0.0	50 <b>W</b>	° 0.00€	0.0	Ø100.00	ý 0 <u>9</u> 0			
0.16	0.0	.00	80.00	<sub>e</sub> 3.3	″83 <sub>6</sub> 3€/°	<b>©</b> 0.0	0'		
0.08	0.0	0.0	√ 10.0°	<b>₽</b> 0.0 ♥	10.0	$\sim 0.0$	? . <b></b>		
0.06	0.0	× 0.0, 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	<b>3</b> .3	) 0.Q	<b>3.3</b>	J 9:07	_~~		
results are average behav. abnorm. =	.~		L j	~~ ~~					

# C. Validity Criteria

C. Validity Criferia.

The contact and oral test are considered valid as the control protality in each case was \le 10\% and the LD<sub>50</sub> values obtained with the required ranges.

# D. Biological Endpoints Derived

An overview of the endpoints derived for both acute contact and acute oral toxicity test is given below:

Acute contact toxicity test Contact LD <sub>5(1,24h)</sub> of BYI 02960: > 200.0 μg a.i./bee
Contact LD (48h) of BYI 02 60: > 200.0 μg a.i./bee
Contact I 0 50 (72h of B VI 0 2 960: 158.4 μg a.i./bee
) College 22 30 (100) C1 22 30 122 10 Mg Will CV
Contact NOED (72h) of BYI 02960: 25 µg a.i./bee
Acute oral toxicity test Oral LD <sub>50 (24%)</sub> of EVI 02960: 1.3 μg a.i./bee
Ocal LD <sub>3</sub> Q <sub>48 h</sub> of SYI 02960: 1.2 μg a.i./bee
Gral NOED (48h) of BYI 02960: 0.68 μg a.i./bee
Behavioural Amormalities: Discoordinated movements and apathy occurred in both toxicity tests.

The contact  $D_{50}$  values (24, 48, 72 and 96 h) of the test item BYI 02960 were determined to be >200, >200, 158 and 122.8  $\mu$ g a.i./bee, respectively. The oral LD<sub>50</sub> values (24 + 48 h) of the test item BYI 02960 were calculated to be 1.3 and 1.2 μg a.i./bee, respectively.

Behavioural abnormalities such as dis-coordinated movements and apathy occurred in both toxicity tests.

Report:	KIIA 8.7.1/02;	(2010)	~ 6)
Title:	Effects of BYI 02960-difluo	( )	ne (Scute Contact and Oral)
	on Honey Bees (Apis mellife	era L.) in the Laborato	ry <sup>©</sup>
Report No:	60291035	ي ا	
Document No:	M-398557-01-2		
<b>Guidelines:</b>	OECD Guideline 213		
	OECD Guideline 214		
<b>Deviations:</b>	For the contact test, a 501	droplet was chosen	(for any of the treatments)
	in deviation to the guidelin	ie recommendation o	f1µQt⁄ 🎺 ৺ "Հ
GLP:	Yes (certified laboratory)	~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the effects of BY1 02960 difluor bethylaming-furanone on

honey bee (Apis mellifera) after oral or contact exposule. The oral dose response test 30 adult Pemale worker honey bees per dose level were exposed for 48 hours to doses of 81.5, 54.3, 26.8, 12.7 and 6.7 µg a.i./bee by feeding values based on the actual intake of the test item). For the contact oxicity test 30 worker bees per dose level were exposed for 48 hours to doses of 100.0, 50.0, 25.0, 12.5 and 6.3 per bee by topical applications

In addition, negative controls [oral. a) water/sugar and b) acetone/sugar; contact: a) tap water and b) acetone] and a toxic reference (Dimethoate; 460 g/L cominal) at nominal rates of 30, 0.15, 0.08 and 0.05 µg dimethoate/bes. Mortality and abnormal behaviour were recorded 4, 24 and 48 hours after test start, respectively. The LD (48h) of the test item was determined to be > 100.0 µg a.i./bee (highest dose level tested) in the contact toxicity, test and  $> 81.5~\mu g$  and /bee in the oral toxicity test, respectively. No test item related behavioural abnormalines occurred petther in the contact nor in the oral toxicity test.

### A. Materials

### 1. Test material

BY102960-diffuoroethyl-amino-furanone (BCS-CC98193) Test item:

Pure metabolite Solid, brown Chemical state and description Solubility: Stability of test compound: Batch No.:

99.2% w/waccording to certificate of analysis

In acetone: not indicated

Expiredate: 04.05.2013, when stored at  $+5 \pm 5$ °C in original

container in the dark

Control

Oral Test: a) 50 % (w/w) aqueous sugar solution (in tap water);

b) 50% (w/w) sugar solution (45% water, 5% acetone, 50% sugar) a) Tap water with 0.5% Adhäsit\* (applied after anesthetization with Contact Test:

b) Acetone (applied after anesthetization with  $\bigcirc O_2$ )

\* (Adhäsit improves spreading of the test droplet on the water

repellent hairs on the thorax of bees)

**Wetting Agent** 

Name: Batch No .:

Analytical content of active ingredient: Manufacturer:

Spiess-Urania Chemicals GmbH,
Heidenkampsweg 7.7, 2009 Hamburg, Gormany
Expiry Date: 12/2011, when stored in objeinal container, at room temperature (20 ± 5°C) in the dark

0.5%

em accordin Storage:

Target Amount in this Study:

**Reference Item** 

The information concerning the reference from according to the substance container label and data sheet:

Perfekthion EC (BAS 152 IV I)

BASSF A@Agric@tural Center Limburg@hof, D-6711-Emburgerhof Manufacturer:

Batch No .:

Analytical content of active ingredi nominal: Dimethoate: 400 g

analyzed Dimethoate: 414.8 g/K

Certificate of Analysis Study Control

Type of formulation: Aggregate State at Room Kiauid ₽

Colour: Density:

Solubility: Topiry date: October 07/2/2011 Stability:

orage: in original container in refrigerator (≤10 °C), in the dark

2. Test organisms

Species:

Common name

Age or developmental stage

start:

olonies, disease-free and queen-right, bred by IBACON Source

B. Study design and methods

Test units were stainless steel chambers of 10 cm x 8.5 cm x 5.5 cm (length x width x height), the front side was a reprovable glass sheet, the bottom was perforated with 98 ventilation holes (Ø 1 mm), the inner walls were lined with filter paper.

10 bees were used per replicate unit, 3 replicates per treatment group (i.e. 30 individuals per treatment group).

Exposure time for both tests was 48 hours.

Food was commercial ready-to-use syrup (Apiinvert; 30% Saccharose, 31% Glucose, 39% Fructose).

Bees in the oral test were starved for 20 minutes prior to test start.

Bees in the contact test were anesthetized for *ca*. 20 seconds with CO<sub>2</sub> until they were completely immobilized immediately before application (only in the contact test).

Control:

a) CO<sub>2</sub>/tap water + Adhäsit<sup>2</sup>; Contact test: b) CO<sub>2</sub>/acetone

Aqueous sugar solution + acctone

3 µga.i./bee

methoate per bee b Aqueous sugar Oral test: a) Aqueous sugar solution;

Test item:

Contact Test:

100.0, 50.0, 25.0 Nominal dosage and 6.3 mg

Oral Test:

Nominal dosage

Measured dosage

Toxic reference item:

Contact test:

Nominal dosage

Oral Test:

0.15, 0.08 and 0.05 Nominal dosage

Measured dosage

Application of the test item in the contact test

Bees were anaesthetized with OO2 in the contact test. A single 5 at droplet of the test item BYI 02960difluoroethylamino-furanone in amappropriate carrier acetone) was placed on the dorsal bee thorax using a Burkard - Applicator. For the controls, one 5 or L droplet of a) tap water with 0.5% Adhäsit and b) pure acetone was used, respectively. The reference item was also applied in a 5 µL droplet (dimethoate made up in acetone)

A 5 μL droplet was chosen in deviation to the guideline regommendation of 1 μL, since a higher volume ensured a more reliable dispersion of the test item; IBACON experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected.

Application of the test itemor the oral test

Appropriate amounts of BYI02960-diffaoroethyl-amino-furanone or reference item dilutions in acetone were mixed will syrup read to-use syrup; Apiinvert, Südzucker, D-97195 Ochsenfurt; composition of the sugar component 30% Sucrose, 31% Glucose, 39% Fructose) in order to achieve the required test concentrations in final dilution of 50% syrup solution (45% water, 50% syrup and 5% acetone (w/w)). For the control the same proportion of syrup, water and acetone was used (solvent control) and similarly, 50% aqueous surup solution was used for the negative control. The treated food was offered in syring which were weighed before and after introduction into the cages (duration of uptake was 3

<sup>&</sup>lt;sup>2</sup> Adhäsit was used to improve the spreading of the test droplet on the bee body. Adhäsit is non-toxic to honey bees.

hours 20 minutes to 6 hours for the test item treatments). After a maximum of 6 hours, the syringes were removed, weighed and replaced by ones containing fresh, untreated food.

The mean target dose levels (e.g. 100 µg a.i./bee nominal) would have been obtained if exactly 20 mg/bee of the treated food were ingested. In practice, uptake of the treated sugar solutions differed slightly from the nominal 20 mg/bee and results are given based on the measured consumption.

# 3. Observation and measurements:

The number of dead bees was determined after 4 hours (first day); 24 and 48 hours (contact and oraclest). Behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after hours (first day); 24 and 48 hours (contact and oral test).

### Result evaluation:

Results obtained from the bees treated with test item were compared to those obtained from the toxic standard and the controls.

The contact and oral LD<sub>50</sub> of the reference item were estimated according to moving average computations (Thompson and Weil, 1992).

If necessary, the LD<sub>50</sub> calculation was carried out taking into account the mortality data corrected by control mortality using Abbott's formula 1925.

The software used to perform the statistical coalysis was ToXRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

### RESULTS AND DISCUSSION

### A. Environmental Parameters

Measurements of climatic parameters during the test are summarized as follows:

Test environment:

Test temperature:

Relative with humidity:

Light intensity:

Incubator

24 25°C

To 86 %

Darkpress (except during observation)

Ventilation: Ventilation by avoid possible accumulation of pesticide vapour Recording: Ventilation by avoid possible accumulation of pesticide vapour Test conditions were continuously recorded with electronic data

logger and documented in the raw data

# B. Biological, Findings

Observations:

Oral Test:

In the oral toxicity test, the maximum frominal dose level of BYI02960 - difluoroethyl - amino - furanone (i.e. 100 µg a i./beep was not achieved, because the bees did not ingest the full volume of treated sugar solution, even when offered over a period of 6 hours. The actual intake at the 100 µg a.i./bee - treatment level resulted on average in 81.5 µg a.i./bee. Oral mean doses of 81.5, 54.3, 26.8, 13.7 and 6.7 µg a.i./bee resulted in no mortality in any of the dose levels. No mortality occurred in the solvent control group or in the water control group (50% sugar solution),

### Table: Mortality and behavioural abnormalities of the bees in the oral toxicity test

	after 4	hours	after 24	hours	after 4	8 hours
	mortality	behav.	mortality	behav.	mortality	behav.
		abnorm.		abnorm.		abnorm.
consumed	mean	mean	mean	mean	mean	mean
μg a.i./bee	%	%	%	%	%	<b>%</b>
test item						0.0 0.0 0.0
81.5	0.0	0.0	0.0	0.0	0.0	<b>₩</b> 0.0
54.3	0.0	0.0	0.0	0.0	0.0	0.0
26.8	0.0	0.0	0.0	0.0	0.0	0.0
13.7	0.0	0.0	0.0	Q. (b)	0.0.0 0.2.0	0.0
6.7	0.0	0.0	0.0	△0.0	0.4Q,"	<u></u> ° 0.0 🞸
water control	0.0	0.0	0.0	0.0	~0.0 €	J 0.0 ♥
solvent control	0.0	0.0	0.0	0.0 0	©0.0 ×	<b>,000</b> (
reference item						
0.30	16.7	43.3	96.₹	© 0.0 ©	1400.0	0.0
0.14	3.3	20.0	<b>76</b> .7	y 0.0 y	<b>≈</b> 83.3 €	, OO) ,
0.08	0.0	0.0	<b>@</b> ₹0.0,°~,"	_3Ø'	<b>20.0</b>	<b>7</b> 0.0 S
0.05	0.0	0.0	Q 0.0 ×	<b>.</b> 0.0 ~	3.39	0.0

behav. abnorm. = behavioural abnormatives

Contact Test:

At the end of the contact toxicity test (48 hours after application) one single bee field in the 25.0 µg a.i./bee dose group (i.e. 3.3% mortality). At the other dose levels (100 n 50 n 12 7 6.3 µg a.i./bee) no wortality  $6.3~\mu g~a.i./bee)$  no prortation occurred. No mortality occurred in the states of th Adhäsit) and there was \$3% mortality in the solvent control group (acetone).

No test item induced behavioural effects were observed any time in the contact toxicity test. Adhäsit) and there was \$2.3% mortality in the solvent control group (acetone).



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

	after 4	hours	after 24	4 hours	after 4	8 hours	_ 0
	mortality	behav.	mortality	behav.	mortality	behav.	
		abnorm.		abnorm.		abnorm.	
dosage	mean	mean	mean	mean	mean	mean	
μg a.i./bee	%	%	%	%	%	<b>%</b>	
test item						4	
100.0	0.0	0.0	0.0	0.0	0.0	€ 0.0	
50.0	0.0	0.0	0.0	0.0	0.0	0.0	
25.0	0.0	0.0	0.0	0.0	3.3	0.0	1 2 5 4
12.5	0.0	0.0	0.0	<b>Q</b> .0	00	0.0 0.0 &	
6.3	0.0	0.0	0.0	<u>⊿</u> ©0.0	<b>1</b> 000	。 0.0°	
water control	0.0	0.0	0.0	o.0 °	~ 0.0 °	( ( ( ( ( ( ( ( ( ( ( ( ( ( ( ( ( ( (	
solvent control	0.0	0.0	0.0	0.0	@° 3.3°≫	$\sim 0.0$	
reference item						Ţ F	
0.30	0.0	23.3	<u>8</u> 6.7 a	0.00	Ø3.3 °	, Q.0	
0.20	0.0	3.3	83.3 Y	6,7	83.3	8.7 <sub>4,1</sub>	
0.15	0.0	0.0	(T) 3,3		, 16 <b>7</b>	3.3	
0.10	0.0	0.0	Q.O' ,	0.0	\$4.3 C	3.3	L. W
results are averages t	from three repl	icates (tell be	ees each) per a	sage/control			
		Q'	Ö Ö	ð é			**Y
behav. abnorm. = be	havioural abno	rmalities 🎺				0, %	<u> </u>

No test item induced behavioural effects were observed at any time in the oral toxicity test.

# C. Validity Criteria

The validity criterion of control mortality <10% is fulfilled the validity criterion regarding the performance of the toxic reference is fulfilled for both contact and oral toxicity test.

### CONCLUSION

The toxicity of BYI 02960-difluor bethylamino furanone was tested in both an acute contact and oral (dose response) toxicity test on honey bees. The LDs (48h) of the test item was determined to be  $> 100.0 \mu g$  a.i./bee (highest dose level tester) in the contact toxicity test and  $> 81.5 \mu g$  a.i./bee in the oral toxicity test, respectively. Notest item related behavioural abnormalities occurred either in the contact or in the oral toxicity test.

Report:	XIIA &7.1/03
Title:	Effects of BX 0296@hydroxy (Acute Contact and Oral) on Honey Bees (Apis
Title:	mellifera Ly in the Laboratory
Report No:	63901035 × C
Document No: @ "	
Guidelines:	OECD Guideline 213
	QECD Guideline 214
Deviations, S	For the contact test, a 5 µL droplet was chosen (for any of the treatments)
Deviation, and a	in deviation to the guideline recommendation of 1 μL
GLP ST ST	Yes (certified laboratory)

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the effects of BYI 02960-hydroxy on the honey bee (Apis mellifera) after oral or contact exposure.

In the oral limit test 50 honey bees (adult female worker bees) were exposed for 48 hours to a single dose of 105.3 µg a.i./bee by feeding (value based on the actual intake of the test teem). For the contact limit test 50 worker bees were exposed for 48 hours to a single dose of 100.0 µg a.i./bee by topi application.

In addition, negative controls [oral: a) water/sugar and b) weetone/sugar: Contact: a) take water and acetone] and a toxic reference (Dimethoate; 400 g/L nominal) at nominal rates of 050, 0.05 µg dimethoate/bee were tested. Mortality and abnormal behavour were recorded hours after test start, respectively.

The LD<sub>50</sub> (48h) of the test item was determined be  $\gtrsim 00.0$ a.i. Dee in the contact toxicity test and > 105.3 µg a.i./bee in the oral toxicity test.

No test item related behavioural abnormalities occurred in the contact test. Que bee showed dis coordinated movements during the 4 hour assessment before dying in the oral toxicity test.

# MATERIAL AND METHODS

### A. Materials

1. Test material  Test item: Type: Chemical state and description: Batch No.: Purity:  Purity:  BYI 02960-hydroxy (BC8-CQ74364) Pure metabolite Solid brown SES 11215-7-10 95.5% www according to certificate of apalysis	coordinated movements during the raises smell goerores dy may, in the star to give the
A. Materials  1. Test material  Test item:  Type:  Chemical state and description:  Batch No.:  Purity:  Purity:  BYI 02960-hydroxy (BCS-CQ74364)  Pure metabolic  Solid brown  SE 11215-7-10  95.5% www according to certificate of apalysis	
A. Materials  1. Test material  Test item:  Type:  Chemical state and description:  Batch No.:  Purity:  Purity:  BYI 02960-hydroxy (BCS-CQ74364)  Pure metabolic  Solid brown  SE 11215-7-10  95.5% www according to certificate of apalysis	MATERIAL AND METHODS Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q
Type: Chemical state and description: Batch No.: Purity:  95.5% Www according to certificate of apalysis	
Type: Chemical state and description: Batch No.: Purity:  95.5% Www according to certificate of apalysis	1. Test material
Chemical state and description:  Solid brown  Batch No.:  Purity:  95.5% www according to certificate of apalysis	1 cst nem.
	Batch No.: SE\$ 11215-7-100 & C
Solubility: . O O - In acetone: soluble &	
Solutionity.	Solubility: In according to the standard of t
Stability of test compound: Expiry date $95.12.2012$ , when stored at $+5 \pm 5$ °C in original container the dark	Stability of test compound. See Expiry date: 45.12.2012, when stored at +5 ± 5 °C in original

Control

Oral Test: % (♠ w) aq@ous sugar solution (in tap water); 50% (w/w) sugar solution (45% water, 5% acetone, 50% sugar)

©a) Tap water with 0.5% Adhäsit\* (applied after anesthetization with Contact Test:

hacetone (applied after anesthetization with CO2)

(Adhant improves spreading of the test droplet on the waterrepellent hairs on the thorax of bees)

Wetting Agent

Name: Batch No .: 0180201

Analytical content of act 190 g/L Marlopon (nominal) Manufactu@r: Spiess-Urania Chemicals GmbH, Heidenkampsweg 77, 20097 Hamburg, Germany

Expiry Date: 02/2013, when stored in original container, at room

temperature ( $20 \pm 5$ °C), in the dark

0.5%

#### Reference Item

The information concerning the reference item according to the substance container label and data sheet:

Name: Perfekthion EC (BAS 152 11 I)

Manufacturer: BASF AG, Agricultural Center Limburgerhof, D-67114

Limburgerhof

Batch No.: 90924-06

Analytical content of active ingredient: nominal: Dimethoate: 400 g/L

analyzed: Dimethoate: 414.8 g/L according to certificate of analysis

Certificate of Analysis Study Code: 346282\_32

Type of formulation: EC

Aggregate State at Room Temperature: Liquid
Colour: Blue
Density: 1.074 g/cm³
Solubility: In water: of

Solubility: In water: eroofsifiable Stability: Expiry date: October 07, 2011

Storage no original container, in refrigorator (\$10°C), in the dark

## 2. Test organisms

Species: Apis mellifer a carnica L

Age or developmental stage at test stage. Adult female worker bees

Source: Ploney see colories, dispase-fice and queen-right, bred by

IBACON

### B. Study design and methods

1. In life dates: 40 to 15 May, 2011

#### 2. Experimental treat@ents

Test units were stabiless steel chambers of 10 cm x 8.5 cm x 5.5 cm (length x width x height), the front side was a removable glass sheet, the ottom was perforated with 8 ventilation holes (Ø 1 mm), the inner walls were lined with filter paper.

10 bees were used per replicate unit, 5 replicates per reatment group (i.e. 50 individuals per treatment group).

Exposure time for both tests was shows

Food was commercial ready-to-use symp (Aprinvert 30% Saccharose, 31% Glucose, 39% Fructose).

Bees in the oral test were starved for 20 minutes prior totest start.

Bees in the contact test were an esthetized for ca. 20 seconds with CO<sub>2</sub> until they were completely immobilized immediately before application (only on the contact test).

Control:

Contact lest: a) CO Tap water + Anhäsit³; b) CO2/acetone

Oral test: (a) Aqueous sugar solution; b) Aqueous sugar solution + acetone

Test item:

Contact Test:

Normanal dosage 🔊 100.0 µg a.i./bee

Pal Test:

<sup>&</sup>lt;sup>3</sup> Adhäsit was used to improve the spreading of the test droplet on the bee body. Adhäsit is non-toxic to honey bees.

Nominal dosage 100.0 μg a.i./bee Measured dosage 105.3 µg a.i./bee

Dimethoate per bee

0.30, 0.15, 0.08 and 0.05 μg Dimethoate per bee

0.26, 0.16, 0.08 and 0.05 μg Dimethoate per bee

Application of the test item in the contact test:

Bees were anaesthetized with CO<sub>2</sub> in the contact test. Single 5 μL droplet of the test item BY 102960-hydroxy in an appropriate carrier (acetone) was placed on the dorsal bee thorax using a Burkard Applicator. For the controls, one 5 μL droplet of all tap water with 0.5 co Adhasit and b) pure was used, respectively. The reference item was also applied in a 5 μL droplet (dimension to the guideline recomblume ensured a more reliable dispersion of the feature lumes are suitable and no adverse effective.

# Application of the test item in the oral fest:

Appropriate amounts of BYL 02960 hydroxy or reference item dilutions in acetone were mixed with syrup (ready-to-use syrup, Apiinvert, Südzucker, D-97/195 Qensenfart; composition of the sugar component: 30% Saccharose, 21% Ghrcose, 39% Forctose) in order to achieve the required test concentrations in a final dilution of 50% syrup solution 45% water, 50% syrup and 5% acetone (w/w)). For the controls, the same proportion of syrup, water and accorde was used (solvent control) and similarly 50% aqueous syrup solution was ased for the negative control. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was one hour 45 minutes for the test tem treatments). After a maximum of one hour 45 minutes, the uptake was complete and the springes were removed, weighed and replaced by ones containing fresh, untreated food.

The mean larget dose levels (e.g. 100 µg a.i./bee nominal) would have been obtained if exactly 20 mg/bee of the treated food were ingested. In practice, uptake of the treated sugar solutions differed slightly from the nominal me mg/bee and results are given based on the measured consumption.

### 3. Observation and measurements:

The number of dead bees was determined after 4 hours (first day); 24 and 48 hours (contact and oral test). Behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4 hours (first day); 24 and 48 hours (contact and oral test).

#### Result exacutation:

Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the control in both the contact and oral tests.

The contact and oral LD<sub>50</sub> values of the reference item were estimated according to moving average computations (Thompson and Weil, 1952).

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

#### RESULTS AND DISCUSSION

#### A. Environmental Parameters

Measurements of climatic parameters during the test are summarized actollows:

Test environment: Incubator Test temperature: 24 - 25°C Relative air humidity:

Light intensity:

Darkness (except during observation)

Ventilation to avoid possible accomulation of positicide vapour.

Test conditions were continuously recorded with electronic data. Ventilation: Recording:

#### **B.** Biological Findings

Observations:

Oral Test:

In the oral toxicity test, the maximum nominal test level of BYI 62960 by drox (100 ug a.i./bee) corresponded to an actual in take of 105.3 ig a.j. bee. This dose level ded to 4,0% mortality after 48 hours.

No mortality occurred in the solven and in the water control group (50% sugar solution), respectively. One bee in the test from treatment showed a dis-coordinated povement (before dying) during the 4hours assessment

Mortality and behaviourakabnounalities of the bees in the oral toxicity test Table:

				2		
	after 4	hours	after 2		after 48	3 hours
* ¥	mortality	behav.	mortality	) Wehav	montality	behav.
consumed		abnorm.		abnorm.	<b>*</b>	abnorm.
dosage	mean	mean	@mean	mean	S mean	mean
μg a.i./bee 🎺	@ % <sup>O</sup>	Ö% >>	<b>%</b> O'	%	% %	%
test item 4	(		5Q' (			_
105.3	2.0	2,00	<b>4</b> .0	0.0	4.0	0.0
water	0.0	40.0° ~	0.00	~ <b>©</b> .0	0.0	0.0
sõl√ent	0:0,	(F)0.0 \(\sigma^{\text{'}}\)	<b>)</b>	$\bigcirc$ $0.0$	0.0	0.0
reference item	_ (	ř "Č	a, 1.	)"		
0.26	<u>√</u> 40.0∢ \	54.0	, 100.0Q	0.0	100.0	0.0
0.16	8:0	<b>2</b> 0.0	9 <b>2</b>	0.0	98.0	0.0
0.08		0.0	<b>%</b> .0	0.0	0.0	0.0
0.05		0.05	0.0	0.0	0.0	0.0
	A 1	*46.				

results are averages from live replicates (ten bees each) per dosage/control

behavabnorm. = behavioural abnormalities

water = water control; solvent = solvent control

Contact Test:

At the end of the contact toxicity test (48 hours after application), there was 0.0% mortality at 100.0 µg a.i./bee. No mortality occurred in the solvent control group (acetone) and there was 2.0% mortality in the water control group (water + 0.5% Adhäsit).

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

Table: Mortality and behavioural abnormalities of the bees in the contact toxicity test  after 4 hours after 24 hours mortality behav. mortality behav. abnorm.  dosage mean mean mean mean mean mean mean mea	No test item ind	duced behavi	oural effec	ets were obs	erved at an	ny time in the contact toxicity test.
dosage μg a.i./bee         mean μg a.i./bee         mean γ/6						
dosage μg a.i./bee         mean μg a.i./bee         mean γ/6	Tables	Mantality an	d haharian	ual ahnauma	litiaa af th	a bass in the contact toxisity test
dosage   mean   mean	rable:	Mortality an	u benaviou	rai abnorma	inues of the	e bees in the contact toxicity test
dosage   mean   mean		after 4	hours	after 24	hours 🍣	after 48 Sours
dosage   mean   mean		mortality	behav.	mortality	behav.	mortality behav.
dosage   mean   mean			abnorm.		abmorm.	abnorm.
test item  100.0	dosage	mean	mean	mean	mean	mean mean s
test item  100.0	μg a.i./bee	%	%	%	<b>%</b>	
water 0.0 0.0 0.0 0.0 2.0 0.0 0.0 0.0 0.0 0.0	test item			<b>&amp;</b>	, S	
water 0.0 0.0 0.0 0.0 2.0 0.0 0.0 0.0 0.0 0.0	100.0	0.0	0.0	0.0	×0.0	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	water	0.0	0.0	Q. <del>19</del>	$\sqrt[\infty]{0.0}$	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	solvent	0.0	0.0	<b>40.0</b>	1 0.0 Y	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$						
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	0.30	18.0	44.0	D * 92,0 ×	% 6.0 €	7 96.9 24.0 5 5 5
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	0.20	0.0	$0.0  \mathbb{Q}'$	80.0	4.6	84.0 6.0 6.0 ° °
results are averages from five replicates (ten bees each) per do sage/control	0.15	0.0	00	, <b>4</b> 22.0 °	2:0	© 32.0
results are averages from five replicates (ten bees each) per do age/control behav. abnorm. = behavioural abnormalities?			£0.0	2.00	0.0	$\stackrel{\mathbb{Y}}{=} 2 \mathcal{O}^{\mathbb{Y}} \mathcal{O} 0.0 \mathcal{O}^{\mathbb{Y}}$
behav. abnorm. = behavioural abnormalities.	results are average	ges from five	replicates (t	ten bees each	per dosag	ge/control & & &
	behav. abnorm.	= behavioural	åbnormaliti	ies,©		

water = CO<sub>2</sub>/water-treated control solv

## C. Validity Critecia

The validity criterion of control mortality 40% is fulfilled; the alidity criterion regarding the performance of the toxic reference is fulfilled for both contact and or toxicity test, respectively.

### D. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

Contact toxicity test:

Since no mortality occurred in the 100.0 considered to be > 100.0 atment group, the contact LD<sub>50</sub> can be considered to be  $> 100.0 \mu \text{g/a}$ .

Oral toxicaty test:

There was 4.0% mortality in the reatment group, therefore, the oral LD<sub>50</sub> can be considered as >

oral LD<sub>50</sub> © 4 h) values of the reference item (dimethoate) were calculated to be 0.17

The toxlety of BYI 02960-hydroxy was tested in both an acute contact and oral toxicity test on honey bees. The LD<sub>50</sub> (48h) of the test item was determined to be  $\geq$  100.0 µg a.i./bee in the contact toxicity test and  $> 105.3 \mu g$  a.i./bee in the oral toxicity test, respectively.

No test item related behavioural abnormalities occurred in the contact test. One bee showed discoordinated movements during the 4 hour assessment (before dying) in the oral toxicity test.

			-0.5	
Report:	KIIA 8.7.1/04;	(2010)	F	4 . 4)
Title:	Effects of Difluoroacetic acid		t and Oral) on Hor	ney Bees (Apis
	mellifera L.) in the Laborator	у		
Report No:	56331035	٥		
Document No:	M-367915-01-2	A,	Q.	
<b>Guidelines:</b>	OECD Guideline 213	4	"O	
	OECD Guideline 214	4	Q, ,	
<b>Deviations:</b>	For the contact test, a 5 µL/	droplet was ch	osen (for all of <b>P</b> o	e treaments) in
	deviation to the guideline re	commendation	noffyn L 🦟	
GLP:	Yes (certified laboratory)			

#### **EXECUTIVE SUMMARY**

of diffuoroacet The aim of the study was to determine the effects *mellifera*) after oral or contact exposure.

In the oral limit test, 50 honey bees adult female work to bees were sposed or 48 to urs to a single dose of 107.9 µg a.i./bee by feeding (value based on the actual intake of the test item). For the contact limit test 50 worker were exposed for 48 hours to a single dose of 100.0 ig a i oper bee by topical application.

In addition, negative controls (contact rest: a) ap water and b) acctone; oral: a) water/sugar and b) acetone/sugar] and a toxic reference Dimetroate; \$00 g/L nominal) at pominal rates of 0.30, 0.15, 0.08 and 0.05 µg dimethoate/beo were tested.

Mortality and abnormal behaviour were recorded 4, 24 and 48 hours after test start, respectively.

The LD<sub>50</sub> (48h) of the test item was determined to be > 100.0 µg a.i./tee in the contact toxicity test and > 107.9 µg a.i./bee in the oral toxicity test, respectively. Notest item related behavioural abnormalities occurred weither in the contact for in the ord toxicity test.

#### A. Materials

## 1. Test material

Test item:

Diffusoroacette acid (BCS-AA56716)
Pure metabolite
Liduid ... Chemical state and description: Batch No.: **BCOQ5984-1-1** 

.8% w/w according to certificate of analysis

In water: miscible (according to test facility) In acetone: miscible (according to test facility)

Expiry date: 29.07.2010, when stored at  $+25 \pm 5$ °C in original

container in the dark

Control

Oral Test: a) 50 % (w/w) aqueous sugar solution (in tap water);

b) 50% (w/w) sugar solution (45% water, 5% acetone, 50% sugar) a) Tap water with 0.5% Adhäsit\* (applied after anesthetization with

Contact Test:

b) Acetone (applied after anesthetization with  $\bigcirc O_2$ )

\* (Adhäsit improves spreading of the test droplet on the water-repellent hairs on the thorax of beach

repellent hairs on the thorax of bees)

**Wetting Agent** 

Name: Batch No .:

Analytical content of active ingredient: Manufacturer:

Spiess-Urania Chemicals GmbH,
Heidenkampsweg 77, 2009 Hambarg, Goman
Expiry Date: 12/2011, when stored in original container, at room temperature (20 ± 5°C), in the dark
0.59

em according to the substantial container of the substantial container o Storage:

Target Amount in this Study:

**Reference Item** 

The information concerning the reference item according to the substance container label and data

Name:

BASF AS, Agricultural Center Limburgerhof Manufacturer:

Limburgerhof © 9092406

Batch No.:

nominal: Immethoate: 400 g/L Analytical content of active ingredien

analyzed Dimethoate: 404.8 g/L

Certificate of Analysis Mady Code:

Type of formulation:

Aggregate State at Room Colour:

Density: 1.074 g/cm Solubility:

In water: emulsificable Expiry date: October 0 22011 Stability:

torage: in organal containe oin refrigerator (\le 10 °C), in the dark

2. Test organisms

Species:

Common name

Age or developmental stage at Adult female worker bees

start:

Source: folonies, disease-free and queen-right, bred by IBACON

Test units were stainless steel chambers of 10 cm x 8.5 cm x 5.5 cm (length x width x height), the front side was a removable glass sheet, the bottom was perforated with 98 ventilation holes (Ø 1 mm), the inner walks were lined with filter paper.

10 bees were used per replicate unit, 5 replicates per treatment group (i.e. 50 individuals per treatment group).

Exposure time for both tests was 48 hours.

Food was commercial ready-to-use syrup (Apiinvert; 30% Saccharose, 31% Glucose, 39% Fructose)

Bees in the oral test were starved for 20 minutes prior to test start.

Bees in the contact test were anesthetized for *ca*. 20 seconds with CO<sub>2</sub> until they were completely immobilized immediately before application (only in the contact test).

#### Control:

Contact test: a) CO<sub>2</sub>/tap water + Adhäsit<sup>4</sup>; b) CO<sub>2</sub>/acetone

Oral test: a) Aqueous sugar solution: b) Aqueous sugar solution + a etong

Test item:

Contact Test:

Nominal dosage 100.0 µg xi./bee

Oral Test:

Nominal dosage 100 pg an bee

Measured dosage 107.9 μ@a.i./be

Toxic reference item:

Contact test:

Nominal dosage 4,30, 0,20, 0, 15 and 0,10 µg Dimethoate per bec

Oral Test:

Nominal desage 0.30, 0.45, 0.08 and 0.05 µg Dimethoate per bee

Measured dosage 0.23 0.15, 008 and 0.05 ug Dimethoate per bee

Application of the test item in the contact test:

Bees were anaesthetized with CO2 to the contact test. A single  $\frac{1}{2}$   $\frac{1}{4}$ L droplet of the test item difluoroacetic acid in an appropriate carrier (accone) was placed on the dorsal bee thorax using a Burkard - Applicator. For the controls one 5  $\frac{1}{4}$ L droplet of  $\frac{1}{4}$ 0 tap water with 0.5% Adhäsit and b) pure acetone was used, respectively. The reference item was also applied in a 5  $\frac{1}{4}$ L droplet (dimethoate made up in acetona).

A 5  $\mu$ L desplet was chosen in deviation to the guideline recommendation of 1  $\mu$ L, since a higher volume ensured a more reliable dispersion of the test item; IBACON experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected.

Application of the test item in the oral test

Appropriate amounts of difluoreacetic acid or reference item dilutions in acetone were mixed with syrup (teady-to-use sorup (teady-to-use sorup)); composition of the sugar component 30% Saccharose, 31% Glucose, 39% Fructose) in order to achieve the required test concentrations in a final dilution of 50 % syrup solution (45% water, 50% syrup and 5% acetone

<sup>&</sup>lt;sup>4</sup> Adhäsit was used to improve the spreading of the test droplet on the bee body. Adhäsit is non-toxic to honey bees.

(w/w)). For the controls, the same proportion of syrup, water and acetone was used (solvent control) and similarly, 50% aqueous syrup solution was used for the negative control. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of upwake was 4.45 hours for the test item treatments). After a maximum of 4.45 hours, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

The target dose levels (e.g. 100 µg a.i./bee nominal) would have been obtained if 20 mg/bee. treated food was ingested. In practice, higher (or lower) dose levels were obtained as the bees higher or lower uptake of the test solutions than the nomina 20 mg/bee.

#### 3. Observation and measurements:

The number of dead bees was determined after 4 hours (first day); 24 and 48 hours (contact and oral test). Behavioural abnormalities (vomiting, apathy intensive cleaning), were day); 24 and 48 hours (contact and oral test).

Result evaluation:

Results obtained from the bees treated with test Obtained from the toxo standard and the controls.

mpared to Prose Results obtained with the bees treated with test item and the referen obtained with the control in both the contact and oral tests.

The contact and oral LD50 values of the reference item were estimated Probio Analysis (according to Finney 1971).

The software used to perform the statistical analy existion 2.10, ® ToxRat Solutions GmbH, © 2009.

# RESULTS AND DISCUSSION

# A. Environmeotal Parameters

Measurements of climatic parameters during the test are summarized as follows:

Tricubator Test enverenment: Test temperature: Relative air humidite

Dorkness (except during Observation) Light intensity:

Wentilmon to ovid possible accumulation of pesticide vapour Ventilation: Recording: Test conditions were continuously recorded with electronic data logger and documented in the raw data

#### B. Biological Finding

Observations:

Oral Test:

In the oral to reity test, no jest item related mortality occurred throughout the entire testing period. No test item induced behavioural effects were observed at any time in the oral toxicity test.



#### Table: Mortality and behavioural abnormalities of the bees in the oral toxicity test

	after 4	hours	after 24	4 hours	after 48	hours
	mortality	behav.	mortality	behav.	mortality	behav.
consumed		abnorm.		abnorm.		abnorm.
dosage	mean	mean	mean	mean	mean	mean
	%	%	%	%	%	96°
test item						4
μg a.i./bee				۵.		Ž <sup>V</sup>
107.9	0.0	0.0	0.0	0.0	0.0	0.0
water	0.0	0.0	0.0	0.0	2.0	0.0
solvent	0.0	0.0	0.0	<b>\$</b>	0.00	0.0
reference item				4	Q" (	
μg a.i./bee			4	20°	A @.	4 Q
0.23	34.0	34.0	100.0	0.00	\$\tag{100.}\text{Q}	<b>0.0</b>
0.15	2.0	8.0	70.0°	<b>20</b> .0 ×	J 72.0	2.0
0.08	0.0	0.0	2.0	0.0	<b>\$</b> 6	0.0
0.05	0.0	0.0	*0 <u>*0</u> *	y 0,0y	<u>≈</u> 0.0 🔏	
esults are average	s from five rep	licates (ten be	ees each) per d	losagecontrol	4 0	
ehav. abnorm. = t	pehavioural ab	normalities 🍃				
water = water cont	rol; solvent = s	solvent contre				
		Q,		ô	2 0	۱۵ ۵
		Ø1		Ţ,		~0 ~0

#### Contact Test:

At the end of the contact toxicity test (48 hours after application), 4% test item related mortality occurred. In the water control (water + 0.5% Adhäsit) and in the solvent control (asetone), 2% and 0% mortality occurred, respectively.

No test item induced behavioural effects were observed at any time in the contact toxicity test.

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

0		9 -	* \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	~		
Ò	after 4		after 24	hogers	after 48	3 hours
	mortality	hehav.	mortality	behav.	mortality	behav.
	·* @	Óbnorm.	, . Č	abnorm.	, O´	abnorm.
dosage	mean (	mean [	Omean 7	mean 2	🔊 mean	mean
_	J % 4		Y %®	0′% <sub>&gt;&gt;</sub>	%	%
test item			`%	Ţ Ş		
μg a.i./bee			~0. <sup>∞</sup> 0	, 10 <sub>h</sub>		
100.0	0.0		Q 4.0 Q	0.0	4.0	0.0
water	0.0	<b>39</b> .0	0.0	<b>W</b> 0.0	0.0	0.0
solvent	0:0	<b>₹</b> 0.0	<b>10</b> .0	<b>∀</b> 0.0	0.0	0.0
reference item		y				
μg⁄a.i./bee			Z, 5,			
	<sub>2,</sub> 42.0	£50.0 @	98,0	2.0	100.0	0.0
0.20	18.0	<b>©</b> 8. <b>Q</b>	86.0	10.0	96.0	0.0
0.30 0.20 0.15	4.0	\$50.0 \$\tilde{\psi}\ 8.0 \$\tilde{\psi}\ 9.0	$\emptyset$ 60.0	0.0	68.0	0.0
0.10	0.0 O	<b>3</b> 0.0	2.0	0.0	12.0	0.0
esults are@werages	fom five replic	atas (ten hees	each) ner dos	age/control	•	•

results are averages from five replicates (ten bees each) per dosage/control

behav. abnorm. Dehavioural abnormalities; water CO<sub>2</sub>/solvent control

#### C. Validity Criteria

The validity criterion of control mortality <10% is fulfilled; the validity criterion regarding the performance of the toxic reference is fulfilled for both contact and oral toxicity test, respectively

### D. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

Contact toxicity test:

Since 4% mortality occurred at the tested dose level of 1000  $\mu$ g a.i./bee, the contact LD $\pi$  can be considered to be  $> 100.0 \mu$ g a.i./bee.

Oral toxicity test:

There was no mortality during the entire test period therefore, the oral  $ID_{50}$  can be considered as  $> 107.9 \mu g \text{ a.i./bee.}$ 

#### **CONCLUSION**

The toxicity of Difluoroacetic acid was fested in both an acute contact and oral toxicity test on honey bees. The LD<sub>50</sub> (48h) of the test item was determined to be 100 ( $\mu$ g at bee in the contact toxicity test and > 107.9  $\mu$ g a.i./bee in the oral toxicity test, respectively. No test item clated behavioural abnormalities occurred either in the contact or fit the shall toxicity test.

Report:	КПА 8.7.1/052 (2010)
Title:	Effects of 6 chloronootinic acid (Acute Contact and Oral) on Honey Bees (Apis
	medifera in the Laboratory of the Laboratory
Report No: Document No:	60281085
Document No:	
Guidelines:	OECD Guiteline 213
Designation of the second of t	QECD Guideline 214 Q
Deviations:	For the contact test, a pull deoplet was chosen (for any of the treatments) in
	deviation to the guideline recommendation of 1 μL
GLP: 🛴 , Ø	Yeocertified laboratory V V

#### EXECUTIVE SUMMARY

The aim of the Mudy was to determine the effects of 6-chloronicotinic acid on the honey bee (Apis mellifera) after oral or contact exposure of the first oral or contact exposure or conta

In the oral limit test 50 honey loes (adult female worker bees) were exposed for 48 hours to a single dose of 107.1 µg a.i. per bee by feeding (value based on the actual intake of the test item). For the contact limit test 50 worker bees per treatment were exposed for 48 hours to a single dose of 100.0 µg a.i. per bee by togreal application.

In addition, negative controls [oral: a) water/sugar and b) acetone/sugar; contact: a) tap water and b) acetone] and a toxic reference (Dimethoate; 400 g/L nominal) at nominal rates of 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee were tested.

Mortality and abnormal behaviour were recorded 4, 24 and 48 hours after test start.

The  $LD_{50}$  (48h) of the test item was determined to be > 100.0 µg a.i./bee in the contact toxicity test and > 107.1 µg a.i./bee in the oral toxicity test, respectively. No test item related behavioural abnormalities occurred neither in the contact nor in the oral toxicity test.



#### MATERIAL AND METHODS

#### A. Materials

#### 1. Test material

6-chloronicotinic acid (AE F161089; BYI 02960-6-CNA) Test item:

Type: Solid, beige Chemical state and description: M12653 Batch No .:

**o**f analysis Purity: 98.8% w/w according to certificate

CAS#: 5326-23-8

In acetone: not indicated Solubility:

Expiry date: 05,07.2012, when s Stability of test compound:

container in the dark

Control

Oral Test:

a) 50% (w/w) aqueous sugar solution (in tab water); b) 50% (w/w) sugar solution (45% water, 5% accione, 50% sugar)

Tap water with 0.5% Adhäsit applied after mesther zation with Contact Test:

CO<sub>2</sub>) Solution with CO<sub>2</sub> Solution with Co<sub>2</sub>

Adhäsit improves spreading of the test Proplet on the water-

repellent hairs on the thorax of Dees

**Wetting Agent** 

Name: Batch No .:

100@/L Marlopon (rominal) Analytical content of activity Spiess-Urania Clomicals EmbH. Manufacturer:

Heiderkampswog 77, 20097 Hamburg Germany

Expire Date: 12/2011, when stored in original container, at room Storage:

temperature (20 ± 5°C), in the dar (0°C)

Target Amount in this Study:

### Reference Item

Reference Item

The information concerning the reference item according to the substance container label and data sheet:

Perfekthion EC (BAS \$32 11 I) Name:

BASFAG, Agricultur Center Limburgerhof, D-67114 Manufacturer:

Limburgerhof

Analytical content of active ingredient Batch No .: 9**9**24-06\$

mominal Dimethoate: 400 g/L

analyæd: Dimethoate: 414.8 g/L according to certificate of analysis

Certificate of Analysis Study Code:

Type of formulation;

Liquid

4**0**√74 g/cm<sup>3</sup>

Type of formulation:
Aggregate State Room Temperature:
Colour:
Density:
Solubility:
Stability: In water: emulsifiable Expiry date: October 07, 2011

Storage: in original container, in refrigerator (≤10 °C), in the dark

#### 2. Test organisms

Species: Apis mellifera L. Common name: Honey bee

Age or developmental stage at test

start:

Source: Honey bee colonies, disease-free and queen light, bred by BACON

Adult female worker bees

### B. Study design and methods

1. In life dates: August 16 to 19, 2010

### 2. Experimental treatments:

Test units were stainless steel chambers of 10 cm x 8.5 cm x 5 cm (length x width) height), the front side was a removable glass sheet, the bottom was perferated with 98 ventilation holes (Q,1 mm), the inner walls were lined with filter paper.

10 bees were used per replicate unit, 5 replicates per treatment group (r.e. 50 individuals per treatment group).

Exposure time for both tests was 48 fours.

Food was commercial ready-to-use syrup (Apinovert; 20% Saccharose, 31% Glucose, 32% Fructose).

Bees in the oral test were starved for 20 minutes prior to test start.

Bees in the contact test were anesthetized for caclo seconds with CQ until they were completely immobilized immediately before application (only in the confact test).

Control:

Contact lest: (a) CO2/tap water + Adhäsit, (b) CO2/acetone

Oral jest: a) Aqueous sugar solution; b) Aqueous sugar solution + acetone

Test item:

Contact Test:

Nomina dosage 6 100 kg a j. De

Oral Test: .«

Nominal dosage Q100 µg a.i./

Measured dosage 🔊 100.1 µg&i./bee

Toxic reference item:

Contact test:

Normal dosage 5 230, 0.20, 0.15 and 0.10 μg Dimethoate per bee

Oral Test

Nomital dosage 0.30, 0.15, 0.08 and 0.05 µg Dimethoate per bee

Measured dosage 0.30, 0.15, 0.08 and 0.05 μg Dimethoate per bee

<sup>&</sup>lt;sup>5</sup> Adhäsit was used to improve the spreading of the test droplet on the bee body. Adhäsit is non-toxic to honey bees.

Application of the test item in the contact test:

Bees were anaesthetized with  $CO_2$  in the contact test. A single 5  $\mu$ L droplet of the test item 6-chloronicotinic acid in an appropriate carrier (acetone) was placed on the dorsal bee thorax using a Burkard - Applicator. For the controls, one 5  $\mu$ L droplet of a) tap water with 1% Adhäsit and by pure acetone were used. The reference item was also applied in a 5  $\mu$ L droplet (dimethoate made up in acetone).

A 5 μL droplet was chosen in deviation to the guideline recommendation of 1 μL, since a higher volume ensured a more reliable dispersion of the test item; IBACON experience has proven that before volumes are suitable and no adverse effects on the outcome of the soldy are to be expected.

Application of the test item in the oral test:

Appropriate amounts of 6-chloronicotinic acid or reference item dilutions in acetone were mixed with syrup (ready-to-use syrup; component: 30% Saccharose, 31% Glucose, 39% Frietose) in order to achieve the required test concentrations in a final dilution of 50% syrup solution (45% water, 50% syrup and 5% acetore (w/w)). For the controls, the same proportion of syrup, water and acetore was used solvent control and similarly, 50% aqueous syrup solution was used for the negative control. The treated food was offered in syringes, which were weighed before and after introduction into the carges (duration of uptake was 3 hours 45 minutes for the test item treatments). After a maximum of 3 hours 45 minutes, the uptake was complete and the syringes were removed, weighed and replaced by ones containing fresh, untreated food.

The target dose levels (e.co 100 µg a.i./bee nominal) would have been obtained if 20 mg/bee of the treated food was ingested. In practice higher dose levels were obtained as the bees had a higher or lower uptake of the test solutions than the nominal 20 mg/bee.

#### 3. Observation and measurements

The number of dead bees was determined after a hours (first day); 24 and 48 hours (contact and oral test). Behavioural abnormalities vomiting, and thy, intensity cleaning) were assessed after 4 hours (first day); 24 and 48 hours (contact and oral test).

Result evaluation:

Results obtained from the bees treated with test item were compared to those obtained from the toxic standard and the controls.

The contact and oral  $LD_{50}$  of the reference item were estimated according to moving average computations (Thompson and Weil 1952).

If necessary the LD% calculation was carried out taking into account the mortality data corrected by control mortality using Abbott formula (1925).

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

#### RESULTS AND DISCUSSION

#### A. Environmental Parameters

Measurements of climatic parameters during the test are summarized as follows:

Test environment: Incubator 24 - 25°C Test temperature: Relative air humidity: 61 to 86 %

Light intensity: Darkness (except during observation)

Ventilation to avoid possible accumulation of pesticide vapour. Test conditions were continuously recorded with electronic data logger and documented in the raw data. Ventilation: Recording:

### B. Biological Findings

Observations:

Oral Test:

In the oral toxicity test, the maximum nominal test level of 6-chloronicotinic acid (100 μg a. bee corresponded to an actual intake of 107. Dag a in bee. This dost level ded to no mortality after 48 fours.

Also no mortality occurred in the solvent control and in the water respectively.

No test item related behavioural Sonormalities Occurred

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

	. 9)			ρ Λ		, X, 1
	after 4	hours	after 2	hour	after 48	3 hours
	mortality	Sbehav.	mortality	behav.	mortality	🎾 behav.
consumed		abnorm.	Ų Į	abnorm,	0, 4	<sup>™</sup> abnorm.
dosage	mean y	ingean 🗸	mean 🦼	9 meado	mean @	mean
		<b>∜</b> % &√		" <u>%</u> "	% % J	%
test item 8						
μg a.i./b <b>ee</b>	10 · · · · ·	_		@w"	% <i>I</i>	
107.19	0.0	JO.0 P	0.0	0.0	> 0.0	0.0
water	0,0° ×	Ø 0.9	©0.0 °°	0.0° ° ×		0.0
solvent	<b>∞0.0</b> ≪	<u>, 0,0 °                                 </u>	©″0.0 </td <td><u></u>\$0.0 △</td> <td>0.0</td> <td>0.0</td>	<u></u> \$0.0 △	0.0	0.0
reference item	& A	36.00				
μg a.i./bee	, *	, S				
0.30	26.0	36.00	>√98.0°>	0.0	100.0	0.0
0.15		<b>19</b> .0 \$	₹ 82¢ <b>)</b>	$\mathscr{O}0.0$	86.0	0.0
$0.080^{\circ}$	0.0	0.0	\$200° \$20°	2.0	20.0	0.0
0.05	Q.0	<sup>♥</sup> 0.0∀	\$\tilde{0}.0.\tilde{\gamma}'	0.0	2.0	0.0

results are averages from five replicates (ten bees each) per dosage/control

Table 6 for details; behav. abnorm behavioural abnormalities

lvent = @lvent@ntrol Q

At the end of the confact to ricity test (48 hours after application), there was 0.0% mortality at 1000 µg a gree. No mortality occurred in the water control (water + 0.5% Adhäsit) and there was 2.0% margality in the solvent control (acetone).

No remarked reactions to exposure of the test item were noted in any of the test bees throughout the duration of the study.

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

							1
	after 4	hours	after 2	4 hours	after 48	hours	
	mortality	behav.	mortality	behav.	mortality	behav.	
		abnorm.		abnorm.		abnorm.	
dosage	mean	mean	mean	mean	mean 🧸	mean	
_	%	%	- 0/0	%	% . <sup>©</sup>	%	
test item			<del>_</del>			, O	ľ . 🔊
μg a.i./bee				Ö	Æ.		
100.0	0.0	0.0	0.0	<b>%</b> 0.0	<b>.</b> 6.0	0.0	<b>9</b> ″ ;
water	0.0	0.0	0.0	0.0	©0.0	<b>19</b> .0 .	
solvent	0.0	0.0	0.0	0.0	Q* 2.Q ·	<u>~0.0 ~</u>	Õ
reference item			20°'			\$;	
μg a.i./bee			W E			\$\ \%	
0.30	0.0	22.0	<b>8</b> 6.0 _ Ø	4.0	\$ 94.0°	<b>30.0</b>	1 .4
0.20	0.0	2.0	∡ 76.0°	#.0 Q	76.9	, 6.0°	
0.15	0.0	0.0	J* 2,0% ,	$\sqrt[8]{0.0}$	″_A2.0 °	6.0	
0.10	0.0	0.0		0.0	$6^{9}2.0$	Ø.0 🛫	
results are averages							
Table 4 for details;				ties, 🔊			
water = $CO_2$ /water-t	reated control;	; sốlvent <del>z</del> yC	O <sub>2</sub> /sodwent co	ptrol			¥

### C. Validity Criteria

The validity criterion of control mortality <10% is fulfilled; the validity criterion of garding the performance of the toxic reference is fulfilled for both contact an Poral toxicity test, respectively.

## D. Biological Endpoints Derived

From the results presented above

### Contact toxicity test:

bee treatment group, the contact LD<sub>50</sub> can be Since no mortality ocean considered as > 1000

bee treatment group, therefore the oral  $LD_{50}$  can be

The toxicity of 6-chloronic and was dested in both an acute contact and oral toxicity test on honey bees. The L  $\mathfrak{D}_{50}$  (48) of the test tem was determined to be  $> 100.0 \, \mu g$  a.i./bee in the contact toxicity test and 107.1 ig a in the oral toxicity test, respectively. No test item related behavioural abnormalities occurred either in the contact or in the oral toxicity test.

Report:	KIIA 8.7.1/06; (2010)
Title:	Effects of 6-chloro-picolylalcohol (Acute Contact and Oral) on Honey Bees
	(Apis mellifera L.) in the Laboratory
Report No:	50911035
Document No:	M-361234-01-2
Guidelines:	OECD Guideline 213
	OECD Guideline 214
Deviations:	For the contact test, a 5 µL droplet was chosen for any of the reatments)
	in deviation to the guideline recommendation of 1 μL
GLP:	Yes (certified laboratory)

#### **EXECUTIVE SUMMARY**

The aim of the study was to determine the effects of BYI-02960 CHMP 6-chlore-picovilal cohol, 6-CPA) on the honey bee (Apis mellifera) after oral or contact exposures.

In the oral limit test 50 honey bees (adult female worker bees) were exposed for 48 hours to a single see dose of 106.7 µg a.i. per bee by feeding (value based on the actual intake of the test item). For the contact limit test 50 worker bees per treatment were exposed for 48 hours to a single dose of 100 pg a.i. per bee for topical application.

In addition, a negative control (water/sugar (oral test); tap water contact test) and a roxic reference (Dimethoate; 400 g/L nominal) agnominal rate of 0.30, 0.1500.08 and 0.00 ug dipethoate/bee, respectively, were tested.

Mortality and abnormal behaviour were recorded 4, 24 and 48 hours after test start respectively.

The LD<sub>50</sub> (48h) of the test item was determined to be \$100.0 µg at bee, in the contact toxicity test and > 106.7 μg a.i./bee in the oral loxicity test, respectively. No test item related behavioural abnormalities occurred neither in the contact nor in the oral toxicity

#### **MATERIAL**

#### A. Materials

#### 1. Test material

6-chloro- (colylacohol (5.6-chloro-3-pyridinemethanol) Test item:

Bure metabolite SolidOvellow@h Type: Chemical stat@and de@r

Batch No .:

6 w/w according to certificate of analysis Purity: CAS#:

Solubility:
Stability of test compound:

Control
Oral Test:
Contact Test: Solubilitv: In water: soft@le

Expry date: Oct. 2009, when stored at room temperature (10 to 30°C) ip original container in the dark

50% (w/w) aqueous sugar solution (in tap water)

Tap water with 0.5% Adhäsit\* (applied after anesthetization with  $CO_2$ 

\* (Adhäsit improves spreading of the test droplet on the waterrepellent hairs on the thorax of bees)



Wetting Agent

Name: Adhäsit Batch No .: 0150207

Analytical content of active ingredient: 100 g/L Marlopon (nominal) Manufacturer: Spiess-Urania Chemicals GmbH,

Heidenkampsweg 77, 20097 Hamburg, Germany

Expiry Date: 12/2009, when stored in original container, at room temperature (10 to 2000) in the Storage:

temperature (10 to 30°C), in the dark

Target Amount in this Study: 0.5%

Reference Item

Reference Item

The information concerning the reference item according to the substance container label and data sheet:

Perfekthion EC (BAS 152 11 I) Name:

BASF AG, Aggioultural Center Limburgerhof D-6 Manufacturer:

Limburgerh

Batch No .: FRE-000627

Analytical content of active ingredient: nominal Dimetlo ate: 400 g/

> analyzed: Dimethoate #22.4 according to certificate

Certificate of Analysis Study Code:

Type of formulation:

Aggregate State at Room Temperature: Liqui Colour: Density:

Solubility: water: Stability:

Expiradate: October 31, 2009 Storage: in original container, in refrigerator (

2. Test organisms

Species:

Common name: Honey bee

Age or developmental

start:

colonies, disease-free and queen-right, bred by Source:

B. Study design and method

2. Experimental treatments

Test units were stainless steel chambers of 10 cm x 8.5 cm x 5.5 cm (length x width x height), the front side was a removable class sheet, the bottom was porforated with 98 ventilation holes (Ø 1 mm), the inner walls were lined with Titer paper.

10 bees were used per replicate unit, 5 ceplicates per treatment group (i.e. 50 individuals per treatment group).

Exposure time for both tests was 48 hours.

Food was commercial ready-to-use syrup (Apiinvert; 30% Saccharose, 31% Glucose, 39% Fructose).

Bees in the oral test were starved for 15 minutes prior to test start in all treatment groups.

Bees in the contact test were anesthetized for ca. 20 seconds with CO<sub>2</sub> until they were completely immobilized immediately before application (only in the contact test).

Control:

Contact test: CO<sub>2</sub>/tap water + Adhäsit<sup>6</sup>

Aqueous sugar solution Oral test:

Test item:

Contact Test:

Nominal dosage 100 µg a.i./bee

Oral Test:

Nominal dosage 100 μg a.i./bee

106.7 µg a.i./bee Measured dosage

Toxic reference item:

Contact test:

Nominal dosage

Oral Test:

Nominal dosage

Measured dosage

Application of the test item in the contact test

test. A single, 5 µL throplet of the test.

A single, 5 µL throplet of the test.

The distribution of the property of the test.

The distribution of the property of the test.

The distribution of the property of the test.

The tap water (dimensions) Bees were anaesthetized with CO2 in the contact test. A single 5 µL droplet of the test item 6-chloropicolylacohol in an appropriate darrier grap water + 05% Adhasit was placed on the dorsal bee thorax using a Burkard - Applicator. For the control, one SuL droplet of tap water containing 0.5% Adhäsit was used. The reference item was also applied in a 5 with tap water (dimethodie made up in tap water containing 0.5% Adhäsit).

A 5 μL droplet was obosen in deviation to the guideline recommendation of 1 μL, since a higher volume ensured a more reliable dispersion of the test item; IFACON experience has proven that higher volumes are suitable and no adverse effects on the officome of the study are to be expected.

Application of the rest item in the oral rest:

Aqueous stock solutions of the test item and reference item were prepared in such a way that they had the respective target concentration of the test item once they were subsequently mixed with sugar syrup at a ratio of 1 + 1. After mixing of these test solutions with ready-to-use sugar syrup (composition of the sugar component 30 % saccharose, 31% glucose, 39 % fructose) the final concentration of sugar syrup in the test item solutions offered to the best was 50 %. For the controls water and sugar syrup was used at the same ratio (1 1/1). The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 2.5 hours for the test item treatments). After a maximum of 2.5 hours, the food uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

<sup>&</sup>lt;sup>6</sup> Adhäsit was used to improve the spreading of the test droplet on the bee body. Adhäsit is non-toxic to honey bees.

The target dose levels (e.g. 100 µg a.i./bee nominal) would have been obtained if 20 mg/bee of the treated food was ingested. In practice, higher dose levels were obtained as the bees had a higher uptake of the test solutions than the nominal 20 mg/bee.

#### 3. Observation and measurements:

The number of dead bees was determined after 4 hours (first day); 24 and 48 hours (contact and test). Behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after day); 24 and 48 hours (contact and oral test).

#### Result evaluation:

Results obtained from the bees treated with test item were compared to those obtained from the standard and the controls.

The contact and oral LD<sub>50</sub> of the reference item were estimated according to proving average. computations (Thompson and Weil, 1952). If necessary, the  $LD_{50}$  calculation was carried out taking into account the mortality data corrected

control mortality using Abbott's formula (1925).

The software used to perform the statistical analysis was Tookat Professional Solutions GmbH, © 2009.

### RESULTS AND DISCUSSIO

marized a Tollow

Measurements of climatic parameters during the test

Test environment:
Test temperature
Relative air humdity

Light:

Light intensity:

Darkness (except during observation)
Vontilation to avoid possible accumulation of pesticide vapour Ventilation: Lest conditions were continuously recorded with electronic data Recording and documented in the Yaw data

## B. Biological Findings

Observations:

ne paximum nominal test level of 6-chloro-picolylacohol (100 μg Oral Test: In the oral toxicity a.i./bee) corresponded to an actual intake of 06.7 og a.i./bee. This dose level led to 2% mortality (1 of 50 beesy after 48 hours.

No mortality occurred in the water corpol (5% aqueous sugar solution).

No test item repited behavioural abnormalities occurred.

Table:	Mortality and						
		hours		4 hours	after 4	8 hours	o° >
	mortality	behav.	mortality	behav.	mortality	behav.	
ingested		abnorm.		abnorm.		abnorm.	
dose	mean	mean	mean	mean	mean	mean	
μg a.i./bee	%	%	%	%	%	<i>®</i> ′ % ≈	, "
test item					4		
106.7	2.0	0.0	2.0	0,0	2.0	0.0	
water	0.0	0.0	0.0	070	0,00	0.0	
reference iter	n			4	NO.		
0.29	78.0	18.0	100.0	0.0	00.0	<b>0.0</b>	Č , C'
0.16	8.0	86.0	94.0	″ 0.0 ペ	y 96. <b>0</b>	$\mathbb{Q}_{0.0}$ $\mathbb{Q}^{v}$	d Ü
0.08	0.0	4.0	38₽	6.0	<sup>°</sup> 50.0	$\bigcirc 0.0$	j' 45
0.05	0.0	2.0	26	© 0.0√	4.0	Ø\$Ô . ´	.4
results are avera	ges from five re	eplicates (ten	begs each)	r dosæge/cor	<b>M</b> OOI O	~ 0	
; water = water/s	ugar treated cor	ntrol			· * A . (		
behav. abnorm. =	= behavioural al	onormalities		. O . K			
		Q					
				IJ <sup>v</sup> ∧			. Q

Contact Test: At the end of the contact toxicity test (48 bours after application), there was 0.0% mortality at 100.0 μg a.i./bee. Also no mortality occurred in the water control (water + 0.5% Adhäsit). No behavioural abnormalities attributed to exposure of the test item to the bees occurred during the experimental time of 48 hours.

There were behavioural abnormalities consistent with the observed toxicity in the reference item test.

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

	. Y O	/Y ~	<b>"</b>	¥	<u> </u>	
	🤊 👸 fter 4	ĥours∜√	after 24	hours Sehav	after 48	8 hours
8	mortality?	behav.	mortadity	Sehavo	mortality	behav.
, <b>©</b>	.O ~ .	abnorm.	, 7	ðabnorm.		abnorm.
dose	mean 🦠	Çmea <b>n</b> Ç '	mean 💸	mean	> mean	mean
<u>μg a.ν./bee</u>		%	~ % % O'	<b>%</b>	%	%
test item	29' 4'					
100.0	6.04 6.04	<u>\$0.0 \$\langle'</u>	*0.0°	0.0	0.0	0.0
water	`Q;@`^	0.0	0.0	<b>1</b> 0.0	0.0	0.0
reference item				<b>∂</b> γ		
0.304	16.0 <sup>©</sup>	\$8.0	<b>₹ 9\$</b> .0 €	$\mathcal{I}$ $0.0$	100.0	0.0
0.20	4.0	⊋ 6.0 <sup>©</sup>	<b>6</b> 0.0	14.0	78.0	0.0
0.15	<b>10.0</b>	00	36:Q	4.0	48.0	0.0
	2.0 \$\sqrt{y}	.%0.0 Q	1000	0.0	10.0	0.0

results are averages from five replicates (top bees each) per dosage/control

behavioural abnormalities; water = CO<sub>2</sub>/water-treated control behav. abnorm

The validity criterion of control mortality <10% is fulfilled; the validity criterion regarding the performance of the toxic reference is fulfilled for both contact and oral toxicity test, respectively.

<sup>;</sup> water = water/sugar treated control

#### D. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

Oral toxicity test:

Since only one bee was found dead during the entire experiment (2% mortality), the oral LD<sub>50</sub> can be considered as  $\sim 106.7$  ug a i /bee

Since no mortality occurred in the 100.0 μg a.i./bee group the contact LD<sub>50</sub> can be considered as > 100.0 μg a.i./bee.

CONCLUSION

The toxicity of BYI 02960-CHMP (6-chloro-picolyfacohol) was tested in both an acute contact toxicity test on honey bees. The LD<sub>50</sub> (48h) of the test item. the contact toxicity test and > 106.7 µg a.i./been the Gral toxicity toxt, respectively. No test item related behavioural abnormalities occurred either in the oral toxicity test.

#### **IIA 8.7.2** Acute contact toxicit

Acute contact toxicity has been evaluated together with acute oral toxicity in the respective studies. Therefore, the study results for both tests are presented begether under Point PA 8.74 above.

#### **IIA 8.7.3** Toxicity of residues on coliage to hopey bees

This is not an EC data requirement. However, a residue study on foliage has been conducted with the formulation BYI 02960 L 2005 following the provisions of OPPTS No. 850.3030. Please refer to the 2011 M-493084-01-1.. Annex III document, **E**IIIA1 10.4.**20**1,

#### Bee brood feeding test ... **IIA 8.7.4**

A bee brood semi-field study has been conducted with the formulated product BYI 02960 SL 200 G, the study is summarised in the Annex 10 for the formulation see report KIIIA1 10.4.7/06, 2012; M-#27438-01-12

#### Effects on non-target terrestrial arthropods **IIA 8.8**

In accordance with the recommendations of the Terrestrial Guidance Document, the toxicity of BYI 02960 to non-target arthropoos was determined using the representative lead formulation (BYI 02960 SL 200 G), for completeness these studies are summarized in this Annex II.

#### IIA **8**8.1 Effects of non-target terrestrial arthropods, artificial substrates

Laboratory tests on artificial substrate (glass plates) have been conducted with the BYI 02960 SL 200 -pecres Aphienus r. on the standar species Apharius rhopalosophi and Typhlodromus pyri. The summaries are presented below.

#### IIA 8.8.1.1 Parasitoid

Report:	KIIA 8.8.1.1/01; (2010)
Title:	Toxicity to the parasitoid wasp <i>Aphidius rhopalosiphi</i> (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) using a laboratory test: BYI 02960 SI, 200 (g/L)
	(Hymenoptera: Braconidae) using a laboratory test; BYI 02960 SL 200 (g/L)
Report No:	CW09/079
Document No:	M-366965-01-2
<b>Guidelines:</b>	MEAD-BRIGGS ET AL. (2000), CANDOLFI ET AL. (2001)
<b>Deviations:</b>	None O S
GLP:	Yes (certified laboratory)

#### **EXECUTIVE SUMMARY**

The objective of this laboratory study was to investigate the lethal and subjectual poxicity of BYI 02960 SL 200 (Sample description: FAR01438-00 (Batch ID: 2009-000253; Material No.: 39718845; Specification No.: 102000021884)) on the parasitoid casp Aphidius Phopatosiphi, when exposed on a glass surface.

The test item was applied on glass plates at nominal rates of 10 20, 40 80 and 160 g a.i./ha, respectively, and effects on 60 adults (Areplicates with 15 wasps per test group) of the parasitoid wasp Aphidius rhopalosiphi were assessed during 24 h after exposure. The control was treated with deionized water (200 L/ha). Dimethoate (0.04 g a.i./ha in 200 L water/ha) was used as 3 toxic reference item. The study was repeated a second time with lower application rates because all tested rates in the first trial showed 100% mortality after 24 h of exposure. In the second study trial, the test item was applied at nominal rates of 0.5, 1.1, 22, 4.7 and 10 g a.i./ha, respectively and mortality was assessed during 48 h after exposure.

At the lowest dose rate of 0.5 g a.i ha, 85% corrected mortality was observed. At all higher test item rates 100% mortality occurred. The LR50 was calculated to be 0.5 g a.i./ha.

Due to the high mortalist in the second trial to assessment of reproductive capacity was performed. Mortality was used to determine the endpoint.

#### MATERIAL AND METHODS

#### A. Materials

1. Test material

Test item: BQI 02960 SL 200 Specification No.: BQI 02960 SL 200 000 21884

Type: Formulated product (soluble (liquid) concentrate)

Chemical state and description Clear brown liquid
Batch No.: 2009-000253
Material number: 79718845
Sample description: FAR 0 438-00
Nominal content of active ingedient: By 02960: 200 g/L

Analytical content of active figredient: 18/1 02960: 17.0% w/w, 199.8 g/L according to certificate of

analysis 1.175 g/mL

Stability of test compound: Approved until 20.03.2010 (storage at +2 °C to +30 °C)



2. Vehicle and/or positive control:

Solvent: No solvent used; deionized water was used as diluent for the test

item and for the reference item

Reference item: Dimethoate EC 400 (analytical content of a.i.: 428.5 g/L.)

3. Test organism

Species: Aphidius rhopalosiphi Parasitoid wasp Common name:

Age: 48 h

48 n Aphidius rhopalosuphi used for testing were supplied by Source of test organism

B. Study design and methods

1. In life dates

November 30 to December 9, 2009

2. Design of the test

Number of test groups:
Number of application rates:

Number of replicates per test group:
Number of larvae/per replicate:

A soluble concentrate formulation of BVI 02960 SL 200 G was tested. The test item was applied at rates of 10, 20, 40, 80 and 160 g a.i. ba on glass plates and the effects on the parasitoid wasp Aphidius rhopalosiphi were compared to those of adeionised water treated control test organisms and the est system.

Mortality of 60 aduds (4, replicates with 45 group) was assessed 2 and 24 h after exposure.

In a second study trial with lower application rates 4. and 10 g a.i./ha mortality was 24 and 48 h after exposure @

Mortality was assessed by recording the condition of the test animals 2, 24 and 48 h (only in second study trial) after application:

- live (alive and apparently unaffected)
- affected (showing reduced co-ordination or any abnormal behaviour)
- but still moving legs or antennae) moribund (unable to walk
- y dead (no longer moving

The computer program SAS Version 9.1 & 2002-2003) was used to perform the statistical analyses.

The mortality data were analysed for significance using the Fisher Exact test (one-sided with Bonferron-Holor adjustment  $\alpha = 0.05$ ), which is a distribution-free test method and does not require testing for nomality or homogeneity prior analysis.



#### RESULTS AND DISCUSSION

#### A. Environmental Conditions

Wasps were kept under conditions which are summarized as follows:

19.5-21.5°C (first trial) Test temperature:

19.5-22.0°C (second trial)

60 - 70 % in the first trial (deviations: decreases <2\Pito 55%) Relative humidity:

60 - 78 % in the second trial

Photoperiod: 16 hours light / 8 hours dark Light intensity 511 - 1009 Lux (first trial)

489 - 661 Lux (second trial)

### **B.** Biological Findings

A summary of effects of BYI 02960 SL 200 on mortality of plates is given in the table below:

Effects of BYI 02960 SL 200 (g/L) on mortality of Table Aphidius chopalosiphi

		≈ ﴿ ﴿		
Test item		BYI 02960 SI	200 (g/D) . 🐇	
Test organism		Aphiaus rhopa	losipud 🧳 🚡	
Exposure on:		Glace plates	losipht of	
Trial 1		Q' & (		
		Mortality after	24 hours [%]	To Volucia
Treatment	g a.i./ha	Uncorr.	24 hours [%]	(P-Vaggle(")
Control	0	197		
Test item	10	1¥00 ≈ .c	₩1100e \	<b>€0</b> :001 sign. ✓
Test item		1100	y 100y 5 4	
Test item	40 80 0	100		<0.001 sign.
Test item	80 0	140 ×	100	<0.901 sign.
Test item	1000	100 🗸 🤍	100 5	\$0.001 <b>Ag</b> n.
Reference item	L <b>Q</b> :04 ~ \\	100%	#40%	
* Fisher`s Exact	est (one sided p-v	values are odfuste	d according to Bool	ferroni-Holm
n.d. not detected,	n.signo not signific	ant, sign, signific	sant S	4)
Trial 2	<b>1</b>			
		Mortality after	48 hours [%]	)
Treatment	g a.i/ha	Uncorr.	Corr	P-Value(*)
Control	10 💝			
Test item	1.1	85.0 💝 🎺 🗸	85.0	<0.001 sign.
Test item	1.1	100	<b>500</b>	<0.001 sign.
Test item ~	2.2 0	1.1000	¥100 <u>~</u>	<0.001 sign.
Test item 🔏	4.7 ° ~	Q100 🖧 👸	1000	<0.001 sign.
Test item ** Reference item	10.0	100 0	100	<0.001 sign.
Reference item  LR <sub>50</sub> : <0.5 g a.i./  * Fisher's Exact t	0.04	100,	~ <b>Q</b> 00	
LR <sub>50;</sub> <b>&lt;0.5</b> g a.i./	ha V		<b>Y</b>	
* Fisher`s Exact t	est (one-si@ed), p@	yalues are adjuste	d according to Bont cant	ferroni-Holm
n d not detected	@sign not signific	ant seen signific	eant	

In the first study rial 147% of the wasps introduced were dead after 24 h of exposure in the control group In all rates of the test tem and the reference item all wasps were dead.

In the second study trial all wasps were found alive in the control group after 48 h of exposure. In the lowest test item rate of 0.5 g a.i./ha 85% of the wasps died whereas in all other rates of BYI 02960 SL 200 g/L as well as in the reference item group all wasps were dead.

#### C. Validity Criteria

C. Validity Criteria	
The validity criteria for	the laboratory method using glass plates of mortality $\leq 13\%$ in the control
•	mortality in the toxic reference are fulfilled.
group, _5070 corrected	moranty in the toxic reference are furnised.
D. Biological Endpoin	its Derived
From the results presen	ated above the following biological endpoints can be derived:
LR <sub>50</sub> :	<0.5 g a.i./ha
CONCLUSION	the laboratory method using glass plates of mortality ≤13% in the control mortality in the toxic reference are fulfilled.  **Ats Derived**  *
The effects of BYI 029	60 SL 200 residues on the survival of the parasitoral was plant that rhopalosiphi
in a laboratory test on g	glass plates can be quantified as an $LR_{50}$ of $< 0.5$ g a.i./ba.
IIA 8.8.1.2 Preda	glass plates can be quantified as an LR <sub>50</sub> of < 0.5 g a.i./ba.
Report:	KIIA 8.8.1.2401; (2010)
Title:	Toxicity to the predatory mite Typhlodromus pyri SCHEUTEN (Acapi, Phytoseiidae)
	using a laboratory gest; BV 0296 CSL 2000 L
Report No:	CW09/0 <b>7</b> 3
<b>Document No:</b>	M-366957-01-27
Guidelines:	BLUEMEL ET AL (2000); CANDOLFI ET AL (2001)
GLP:	Yes (certified laboratory) ( V V V V
EXECUTIVE SUMM	ARY Solve 2007 Sample description; PARQ 438-00 (Batel ID: 2009-001253; Material
The test item BYI 2296	60, SD 200, 8 ample description: FAR01438-00 Batel 1D: 2009-001253; Material

### EXECUTIVE SUMMARY

The test item BYI 05960 80 200 (Sample description: AR01438-09 (Batch 1D: 2009-001253; Material No.: 79718845; Specification No.: 102000023884) was tested under laboratory conditions via residual contact exposure of protonymphs of the predators mite sphlodromus byri to spray residues with rates of 2, 4, 9, 19 and 40 g a.i. Laa, respectively in 200 L deionized water ha applied on glass plates. The control was treated with deion zed water (200 L/ha) Dimethoate & 400 (4 g a.i./ha in 200 L water/ha) was used as a toxic reference item.

Mortality of 100 mites (5 replicates of 20 individuals per test group) was assessed 1, 4 and 7 days after exposure by counting the number of living and deal mites. The number of escaped mites was calculated as the difference from the total number exposed

At the test tem rates of 2 and 40 a.i./ha a conjected portality of 6.3% each was observed. At the higher rates of 9, 19 and 40 gai.i./ha\ a corrected mortality of 25.0, 50.0 and 89.6%, respectively, occurred. The LR<sub>50</sub> was calculated to be 17 g a.i./ha? (95% CI: 13 to 21 g a.i./ha).

#### MATERIAL AND METHODS

#### A. Materials

1. Test material

Test item: BYI 02960 SL 200

Formulated product (soluble (liquid) conceptrate) Type:

Chemical state and description: Clear brown liquid 102000021884 Specification No Batch No.: 2009-001253 Material number: 79718845 Sample description: FAR01438-00 & Nominal content of active ingredient: BYI 02960: 200 g/L

BYI 02960 7.0% w/w Analytical content of active ingredient:

analysis

Density: 1.175 JmL

Stability of test compound: Approved uptil

2. Vehicle and/or positive control

Solvent

item and for the reference

Negative control:

Dimethoate EC Positive control:

3. Test organism

**Species** Proton mphs@ Age

Eggs of the predatory mite were supplied by Source of test organism

# B. Study designand prethods

1. In life dates: 🏚 to February 🏗

Number of test groups.

Number of application rates

Number of replicates per lest group one exposure unit)

Number of larvae/per replicates

The test item (soluble concentrate formulation of BVI 02960 SL 200 (g/L)) was applied onto glass plates at rates of 2, 4, 9, 19 and 40 g a.i./log and the effects on the predatory mite Typhlodromus pyri were compared to those of a deionised water the ated control. A toxic reference (active substance: dimethoate) applied at 4 g a is ha was included to indicate the relative susceptibility of the test organisms and the test system.

### 3. Observation and measurements

Mortality of 100 mites (5 repricates of 20 individuals per test group) was assessed 1, 4 and 7 days after exposure by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed.

#### 4. Statistics

The computer program SAS (Version 9.1.3, 2002-2003) was used to perform the statistical analyses.

The mortality data were analysed for significance using the Fisher Exact test (one-sided with Bonferroni-Holm adjustment;  $\alpha = 0.05$ ), which is a distribution-free test method and does not require testing for normality or homogeneity prior analysis.

The reproduction data were tested for normal distribution using the Shapiro-Wilk test and for homogeneity using the Levene test.

nonnogeneity using the Levene test.

As the reproduction data in this study were not normally distributed the Wilcoxon test one safed with Bonferroni-Holm adjustment;  $\alpha = 0.05$ ) was used.

The LR<sub>50</sub> value was calculated using Probit analysis.

RESULTS AND DISCUSSION

A. Environmental Conditions

Mites were kept under conditions which are summarized as follows:

Test temperature:
Relative humidity:
Relative humidity:
Photoperiod:
Light source

B. Biological Findings

#### **B.** Biological Findings

The mortality / escaping rate in the control exposure units up to day Tafter to atment was 4.0%. A summary of effects of BYI 02960 St. 200 gd and the tox reference on mortality of Typhlodromus pyri exposed on glass fover slides is given below:

Effects of BYI 02960 SI 200 (g/ls) on mortality of Typhadromus pyri **Table** 

(( ))*	~, , o			
Test item		BYI 02960 SL 200	(g/L)	
Test organism		Taphlodromus pyri		
Exposure on:		Class cover slides	4) O'	
* %		Mortality after 7 da	ys [%] 👋	
Treatment	ga.i./ha	Uneorr.	Corg.	P-Value(*)
Control	20 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	40		
Test item			63	0.164 n.sign.
Test item *	40 0	10.0	6.3	0.164 n.sign.
Test item 🔟	9 0 0		25.0	<0.001 sign.
Test item Test item	19 &	<b>9</b> 2.0	50.0	<0.001 sign.
Test item	40 4	90.0 \$ \$9'	89.6	<0.001 sign.
Reference item	40" 50	80,0	79.2	

LR<sub>50</sub>: 17 g a.i./ha; 95 % Confidence Interval: (13,-21) (calculated with Probit analysis)

# C. Validity Criteria

The validity enteria of mortality  $\leq 20\%$  in the control group and  $\geq 50\%$  corrected mortality in the toxic reference are fulfilled (laboratory method with glass plates (BLUEMEL ET AL., 2000)).

<sup>\*</sup> Fisher's Exact test (one-sided) p-values are a Quisted according to Bonferroni-Holm n.sign. not sign cant, sign. significant

#### **D. Biological Endpoints Derived**

From the results presented above the following biological endpoints can be derived:

LR<sub>50</sub>: 17 g a.i./ha (95% Confidence Interval: 13 – 21)

#### **CONCLUSION**

The effects of BYI 02960 SL 200 residues on the survival of the predatory mite *Typhlodropius pyr* in a laboratory test on glass plates can be quantified as an LR<sub>50</sub> of 17 g a.i./ha

### IIA 8.8.1.3 Ground dwelling predatory species

Based on the results of the studies reported under 8.84.1 and 8.8.1.2 no tests with additional species relevant to the use pattern of the product are required.

## IIA 8.8.1.4 Foliage dwelling predatory species

Based on the results of the studies reported under \$8.1.1 and 88.1.2, pe tests of the additional species relevant to the use pattern of the productore required.

# IIA 8.8.2 Effects on non-target terrestrial arthropods in extended lab semi field test

Extended laboratory tests have been conducted with the formulated product BYI 9960 SL 200 G and are filed in the corresponding Annex III document at point IIIA 190.5.2.

In addition, aged-residue stralies have been conducted and are filed in the corresponding Annex III document, at point IIIA1 10.5.3

#### IIA 8.8.2.1 Parasitoid

Please refer to point IIA 8.8.2

#### IIA 8.8.2.2 Predatory mites

Please reser to point IIA 8.8.2.

#### IIA 8.8.2.3 Ground dwelling predator ospecies

Please refer to point II 8.8.2

#### IIA 8.8.2.4 Foliage dwelling predatory species

Please refer to point ILA 8.8.2

## IIA 8.8.2.5 Other terrestical invertebrates

Please refer to point IIA 8.8.2

### IIA 8.9 Frects on earthworms

To assess the impact of BYL02960 on earthworms, laboratory studies were conducted with the technical substance BYI 02960, the formulation BYI 02960 SL 200 G, and the major soil metabolites BYI 02960 difluoroacetic acid and 6-chloronicotinic acid. Acute and chronic laboratory earthworm studies are available for the active substance and both metabolites. The earthworm reproduction study

for the active substance was performed, in accordance with guidelines, with the formulated product BYI 02960 SL 200 G.

#### **IIA 8.9.1** Acute toxicity to earthworms

Additionally studies on s under point 8.14.	oil organisms <i>Folsimia</i> and <i>Hypoaspis</i> have been performed and are reported	
•	exicity to earthworms	»_
Report:	KIIA 8.9.1/01; (2010)	2
Title:	BYI 02960 (tech.): acute toxicity to carthworms (Effenia fetida) tested in artificial soil	Z,
Report No:	LRT/Rg-A-131/09	<b>O</b>
Document No:	M-363742-01-2	,
<b>Guidelines:</b>	OECD-Guideline No. 207	
<b>Deviations:</b>	None S S S S S S S S S S S S S S S S S S S	
GLP:	Yes (certified laboratory)	

#### **EXECUTIVE SUMMARY**

The aim of the study was to determine the acute effects of BXX 02969 (Sample description) TOX 08508-00 (Batch ID: 2009-000299; purity 96.2% w/w) on surv (Eisenia fetida andrei).

Adult earthworms (more than two months old, four replicates of 10) were exposed in an artificial soil system with peat content of 10% over a period of 14 days to concentrations of \$26, 10, 18, 32, 56, 100 mg test item / kg dry soil (1st run) and 178, 316, 562 and 1000 mg test item (kg dry soil (2nd run). In addition a water control was texted.

Mortality and sublethat behavioural effects were used to determine the ondpoints.

The 14-day-LC50 was 1929 mg a r/kg dry soil, the 14-day-NGEC was determined to be < 5.6 mg a.i./kg dr@soil.

### A. Material's

## 1. Test material

rype of test material:
Chemical state and description
Origin Batch No.:
Sample description:
CAS name. Substance, technical Beige powder

200@00023 (Batc Code: BYI 02960-01-03)

T**QX** 08**508**-00 «

2(5H)-foranone 4-[[(6-chloro-3-pyridinyl)methyl](2,2-

difluoroethylamino]-CAS 951659-40-8

4/[[(6-chtoropyridin-3-yl)methyl](2,2-difluoroethyl)amino]furan-IUPAC name:

Purity: Stability of test compound: 96.2**‰** w/w

Expry date: 16.01.2011, when stored at  $\pm 25 \pm 5$ °C



#### 2. Test solutions

Test item mixed with: Artificial soil, 10 % peat

Method of preparation: Each application mixture (5 g) was transferred separately to artificial

soil (595 g dry weight) and mixed

Controls: Water control

Reference substance Chloroacetamide A.R.

#### 3. Test organisms

Species: Eisenia fetida andrei

Common name: Earthworm

Source: In-house lab culture, strain of

Age at study initiation:

More than two months old

Feeding during test: None

Weight at test start: 0.35 g (both runs)

Maintenance of culture:

Temperature:  $22 \pm 2$  Photoperiod: 12 hours light, 12 hours dark

Food: Dried cattle manure at 14 day intervals

#### B. Study design and methods

#### 1. In life dates

November 3, 2009 to January 26, 2010

# 2. Design of biological test

The adult worms used in this study were more than two months old. On the day proor to the beginning of the study, they were transferred from the breeding substrate to an artificial soil (without test item) under the test conditions for acclinatization. Ten earthworms were placed in a randomized procedure in each test container. The average weight was 0.38 g (first and second run).

Earthworms (Eisenid Jetida andrei: more than two months old) were exposed to BYI 02960 (purity 96.2 %) in an artificial soil system over a period of 14 days. Sominal concentrations were 5.6, 10, 18, 32, 56, 100 mg test item/kg dry soil (1st run) and 178, 316, 562 and 1000 mg test item/kg dry soil (2nd run). In addition, a water control was tested.

Each jar (glass jar, 1.5 L) filled with 595 g dry weight test soil (equivalent to 803 g wet weight) served as one replicate 10 worms were used per replicate. The test was conducted with 4 replicates per treatment level. In the controls 8 (four inteach ran) replicates were tested. The test was conducted at 20  $\pm$  2°C and 531 to 586 lux (over both runs) at constant light. The artificial soil contained 10 % peat, 20 % kaolinite clay, 69.7% quartz sand and 0.7% calcium carbonate.

#### 3. Analytical verification

Not applicable.

# 4. Observation and measurements

Seven day after the start of the study, the number of surviving earthworms was determined and returned to the last containers. After 14 days, the weight, abnormal behaviour, observed symptoms as well as the number of surviving earthworms were determined.

Mortality of worms, intoxication symptoms and physical-chemical soil parameters were assessed as indicated below in the result section.



#### 5. Statistical analysis

The LC<sub>50</sub>-values and the 95 percent confidence limits were calculated by Probit-Analysis according to "Maximum-Likelihood" Method (D.J. Finney, 1978). The statistic software used was ToxRatPro Version 2.09.

#### RESULTS AND DISCUSSION

#### A. Physical and Chemical Parameters

The LC <sub>50</sub> -values and the 95 percent con-	fidence limits were calculated by Probit-Analysis according to,
"Maximum-Likelihood" Method (D.J. F	Sinney, 1978). The statistic software used was ToxRatPro
Version 2.09.	
RESULTS AND DISCUSSION	Sinney, 1978). The statistic software used was ToxRatPro
A. Physical and Chemical Parameters	
Table: Properties of the test soil	
Parameter	Start of study  Endor study
First run	
pH value	5.74 \$ 5.73 \$ 5.70\$ \$ 5.60
water content in the	26.4 26.6 25.8 25.8
artificial soil (%)	
WHC <sub>max</sub>	67.7 2 2
WHC <sub>max</sub> (mean)	64.5 0 0 64.5 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
water content as % of WHC <sub>max</sub>	53.0 56.1 547 \$3.9 0
Second run	
pH value	\$5.67 \$ 5.6\$
water content in the	24.9 25.0 24.6 24.7
artificial soil (%)	
WHCmax	578 593 2 503
WHC <sub>max</sub> (mean)	\$7.4 \Q\ \Q\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \
water content as % of WH hax	58.00 56.9

<sup>\*)</sup> taken into account the WHC from the state of

### **B.** Biological Finding

Observations on modality, immobilisation and sublethal intoxication symptoms are listed as follows:

Effects of BVI 02960 on mortality and body weigh chang of Eisenia fetida andrei Table:

Concentration of	% mo	rtality 0	% Weight alteration of
test Gem	mean	$\pm SD$ )	` <b>⊘</b> ″ the survivors
			(mean ± SD)
[mg/kg dry soil] 🙈	🏏 ~day 7🛇 🦠	day 14	day 14
control 🖔	day 75		-3 ± 3
5.6 <sub>@1</sub>			-12 ± 3 *
10		<del>y</del> <del>y</del> 0	-13 ± 3 *
18,3	O 0 Q 4		-17 ± 3 *
32) ** \$6			-20 ± 3 *
\$6			-23 ± 3 *
<u>√</u> 100 ×		$3 \pm 5$	-25 ± 2 *
178	0 5 ± 6	$0^{23} \pm 19$	-25 ± 2 *
316	68 15	$95 \pm 6$	-38 ± 15 *
562	♣ ± 5 ∜	100	n.a.
1000	100 Q	100	n.a.

<sup>\*</sup> statistically different from control Williams-Test ( $\alpha = 0.05$ , one-sided smaller)

No subjethal behavioural changes were observed. No morphological effects were observed.

#### C. Validity Criteria

The validity criterion of control mortality less than 10% is fulfilled.

#### D. Test with toxic reference substance

D. Test with toxic re	
Reference substance:	Chloroacetamide A.R.  18 NOV 2008  13.2 mg/kg  ints Derived  ented above the following biological endpoints can be derived:  effect-concentration)  5.6 mg a.i./kg dry soil  192.9 mg a.i./kg dry soil  (95% confidence limits: 143 30 2760 mg /kg)
Date of most recent tes	st: 18 NOV 2008
Result: LC <sub>50</sub>	13.2 mg/kg
E. Biological Endpoi	ints Derived
From the results prese	ented above the following biological endpoints can be derived:
14-day-results	
NOEC (no-observed-e	effect-concentration) < 5.6 mg a.i. Rig dry soil
	ed-effect-concentration) 5.6 mg a.i.kg dry soil
LC <sub>50</sub>	192.9 mg a.i./kg dry son
	(95% confidence limits: 143, 300 2769 mg/kg)
CONCLUSION	
The acute effect of B	YI 02960 on earthworms Azisenia Jetida andrei Can be quantified as a 14-day-
LC <sub>50</sub> of 192 9 mg a i	/kg dry soil. The highest concentration with to mortality and no soblethat an he set to < 5.6 mg a.l./kg dry soil the lowest concentration tested).
habovioural affacts as	on he set to < 5.6 mg & /kg dw soil the lawest expentition tested)
benavioural effects ca	nn be set to < 5.6 mg/a.1./kg dry soil (the lowest concentration tested).
Danaute	KIIA 8.9.1702;
Report: Title:	BYI 02850-diffuoroacetic acid: acute toxicity to carthworms (Evenia feuda) tested in
Title.	artificial soil,
Report No:	LR@/Rg-AQ35/10
Document No:	M <sup>2</sup> 3688 <u>3</u> 5-01-2
	OECD Guideline No. 207
Guidelines: Deviations:	None S S S S S S S S S S S S S S S S S S S
GLP:	
GLF:	Yes (certified laboratory) 🔊 🗴 🗸 U

### EXECUTIVE SUMMARY.

The aim of the study was to determine the acute effects of BX 02969-difluoroacetic acid (Batch code: BCS-AA\$6716; TOX @889-000, purity 95.8% w/woon survival and growth of earthworms (Eisenia fetida andrei).

Adult earthworms (more than two months old four replicates of 10) were exposed in an artificial soil system with pear content of 10% over a period of 12 days to concentrations of 31.3, 62.5, 125, 250, 500 and 1000 mg test item bg dry soil. In addition a quartz sand control was tested.

Immobilisation and sublishal behavioural effects were used to determine the endpoints.

The 14-day-LC<sub>50</sub> was 1000 mg/kg dry weight soil, the 14-day-NOEC was determined to be 31.3 mg/kg dry weight soil.

#### MATERIAL AND METHODS

#### A. Materials

1. Test material

Test item: Difluoroacetic acid (Code: BCS-AA56716)
Type of test material: Substance technical (pure metabolite)

Chemical state and description: Colourless liquid

Origin Batch number: BCOO 5984-1-1 (Batch code: BCS-AA56216-01-01

Material number: BCS-AA56716
Sample description: TOX 08889-00
CAS#: 381-73-7
Purity: 95.8% w/w

Stability: Expiry date: 29.07,2010, when wored at  $+25 \pm 6$ 

2. Test solutions

Test item mixed with: Quartz sand

Method of preparation: Application multures of 10 g each were transferred separated to

artificial soil 595 g dry weight) and mixed thoroughly using a

laboratory mixer

Controls: Water control

3. Test organisms

Species: Eisenit Jetida and Common name: Earthworm

Source: In-Rouse late culture; strain of

Age at study initiation: Whore than two months of

Feeding during test: No Weight at test start; No 0.3

Maintenance of culture:

Temperature:

Photoperiod:

12 hours light, 12 hours dark

Food:

Doied cattle manure at 14 day into

B. Study design and methods

2. Design of biological test

The adult worms used in this study were more than two months old. On the day prior to the beginning of the study, they were transferred from the breeding substrate to an artificial soil (without test item) under the test conditions for acclimatization. Ten parthworms were placed in a randomized procedure in each test container. The average weight was 0.32 g.

Earthworms (*Eisenia fetida aparei*; more than two months old) were exposed to difluoroacetic acid (code: BCS-A 56716, purity 95,8 % w/w/ in an artificial soil system over a period of 14 days. Concentrations were 31.3 62.5 125, 250, 500 and 1000 mg test item / kg dry soil. In addition a quartz sand control was tested. Each replicate consisted of a jar (glass; 1.5 L) filled with 595 g dry weight test soil (equivalent to 863 g wer weight). 10 worms were used per replicate. The test was conducted with 4 replicates per treatment level. The test was conducted at 20 ± 2°C and 529 lux at day 0, 547 lux at day 7 and 559 lux at day 14 at constant light. The artificial soil contained 10% peat, 20% kaolinite clay, 69.6% quartz sand and 0.4% calcium carbonate.

#### 3. Observation and measurements

Seven days after the start of the study, the number of surviving earthworms and after 14 days, the weight, abnormal behaviour, observed symptoms as well as the number of surviving earthworms were determined. Biological data and physical-chemical soil parameters were assessed as indicated below in the result section.

determined. Biological data and physical elicinical son parameters were assessed as indicated by with
the result section.
4. Analytical verification
Not applicable
5. Statistical analysis
The LC <sub>50</sub> -values and the 95 percent confidence limits were calculated by Probit-Amilysis according to
"Maximum-Likelihood" Method (D.J. Finney, 1978). The statistic software used was ToxRatPro
Version 2.09.
the result section.  4. Analytical verification  Not applicable  5. Statistical analysis  The LC50-values and the 95 percent confidence limits were calculated by Probit-Analysis according to "Maximum-Likelihood" Method (D.J. Finney, 1978). The statistic software used was ToxRatPro Version 2.09.  RESULTS AND DISCUSSION  A. Physical and Chemical Parameters  Table: Properties of the test soil  Parameter  Of study  Of study  PH value  6.18  6.25  6.00
A. Physical and Chemical Parametos 📡 🔊 🔊 🔊 💆 💆
Table: Properties of the testing of
Parameter Start Endy of study
[pii value 0.25 ]
water content in the artificial spil (%) 25.9 26.0 25.2 25.3
WHC contains a contain the distribution of the contains a contain the contain the contains a contain the contains a contain the contains a contain the contain the contains a contain the contains a contain the contain the contains a contain the contain
WHC <sub>max</sub> (mean) 565.4 50.7 51.1
water content as % of WIC <sub>max</sub> 53.3 50.7 51.1

<sup>\*)</sup> taken into account the WHO max from the start of the study

# B. Biological Findings

Observations on immobilisation and subjectival intoxication comptons are listed as follows:

Effects of BCS-AA56/16 on mortality and body weight chance of Eisenia fetida andrei

		77
Concentration of	🗸 💇 % moortality 🗸 💍 🦠	% weight alteration of
		the survivors
(nominal) $\mathcal{Q}$		$(mean \pm SD)$
(mg/kg dry soil)	day 7 day 140	day 14
contr <del>o</del> l,		+6 ± 3
34.3		$+5 \pm 3$
<sub>2</sub> ,62.5		-2 ± 4 *
√ 125 °		-4 ± 4*
250	2.5 ± 5	-6 ± 3*
500		-8 ± 4*
1000 🔎 "		-11 ± 3*

<sup>\*</sup> statistically different from Ontrol Williams Test ( $\alpha = 0.05$ , one-sided smaller)

No morphological and behavioural effects were observed.

## C. Validity Criteria

The validity criterion of control mortality less than 10% is fulfilled.

#### D. Test with toxic reference substance

Reference substance: Chloroacetamide A.R. Date of most recent test: February, 2009

Result: LC<sub>50</sub>: 13.2 mg Chloroacetamide A.R. /kg dry weight soil (95 %

confidence limits: 12.0 - 14.5 mg/kg).

#### E. Biological Endpoints Derived

From the results presented above the following biological adpoints can be derived:

LC50 (14 day)> 1000 mg/kg dry weight soilNOEC31.3 mg/kg dry weight soilLOEC62.5 mg/kg dry weight soil

#### **CONCLUSION**

The acute effect of BYI 02960-difluoroacetic acid (BCS-AS56716) on earthworms (Eigenia fetida andrei) can be quantified as a 14-day-LC5007 > 1000 mg/kg dry weight soil. The highest concentration with no mortality and no sublethal behavioural effects (NOEC) can be set to 31.3 mg/kg dry weight soil.

The following study was performed for the registration of accaming and is the property of Nippon Soda Co. Ltd, access to the study has been granted. The study has been evaluated during the Annex I inclusion of acetamiprid, therefore only a very short summary of the study conclusion is repeated here. Note- the test compound IC-0 is identical to 6 ENA.

Report:	KUIA 8.03/03; P.M. 1999©
Title:	10-0: Acute toxicity to Parthyorms (Eisenia fortida)
	C007758 (Study report number: 282/575)
Document No:	MKU96591-01-1
Guidelines:	QECD-Guideline No. 207 (1984)
I	None S S S
GLP:	Yes certified laboratory

#### EXECUTIVE SUMMARY

The aim of the study was to determine the acute effects of 6-chloronicotinic acid (Lot No. MF-4267; code: BYI 02960-6-CNA (C-0); purity 99.7%) is earthworms (Eisenia foetida) in artificial soil.

Following a preliminary range anding study 60 earthworms (six replicates of 10 worms) were exposed to a single concentration of 4000 mg/kg of soil for a period 14 days. The number of mortalities was determined after 7 and 14 days of exposure. A positive control study using chloroacetamide, conducted approximately every 6 months is reported for reference purposes.

Mortality and body weight strange were used to determine the endpoints.

The 14-Day Los for the test material to earthworms (*Eisenia foetida*) based on nominal test concentrations was greater than 1000 mg/kg. Correspondingly the No Observed Effect Concentration (NOEC) was 1000 mg/kg.

The result of the positive control study gave a 14-Day LC<sub>50</sub> for chloroacetamide (positive control) of 23 mg/kg with 95% confidence limits of 22 - 24 mg/kg.



#### **IIA 8.9.2** Sublethal effects on earthworms

To investigate sublethal effects of BYI 02960 on earthworms a chronic study on earthworms was performed with the formulated product BYI 02960 SL 200G instead of technical substance. summary of this study is provided in the Annex II as representative for the toxleity to the active substance. Additionally studies on metabolites are included in this section.

Donoute	VIIA 9 0 2/01. T. 2010	,
Report:	KIIA 8.9.2/01; T.; 2010	(
Title:	BYI 02960 SL 200 G: Effects of survival, growth and reproduction on the	<b>.</b> @
	earthworm Eisenia fetida tespod in artificial soil	
Report No:	LRT-RG-R-76/09	V V
Document No:	M-392964-01-2	
Guidelines:	ISO 11268-2, 1998 (E) and QECD 222 (2004)	e (
<b>Deviations:</b>	None A O Q Q O O	7)
GLP:	Yes (certified laboratory)	,

#### **EXECUTIVE SUMMARY**

The aim of the study was to determine the effects of By 02960 102000021884 (Sample description: FAR01438-00, Batch ID: 2009-001253; Material No.: 79718845); (purity 199.8 g BYI 02960/L = 17.0% w/w) on growth and reproduction of earthworms (Eisenia fetida andrei).

Earthworms (approximately 7 pointh old, 8 x 90 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed in an artificial soil system over a period of 56 days to nominal concentrations of 8.9, 15.8, 28.1, 50 and 850 mg product kg dry weight soil. In addition a water Control was tested. The test frem was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined. The overall NOEC was determined to be 8.9 m@product/kg dry w eight soil Based on reproduction.

## A. Materials

#### 1. Test material

Test item: Type of test material: Formulated product Specification No.: 102000021884 Material number: Sample description; Batch No .: 2009-001253 Nominal content of active in redient: BX 02960: 200 g/L

Analytical content of active ingredient: BYI 02960: 17.0% w/w, 199.8 g/L according to certificate of

analysis

1.175 g/mL at 20°C

Expiry date: 2010-03-20, when stored at 25±5 °C



#### 2. Vehicle and/or positive control

Test item mixed with: Water

Controls: Water control as negative control

#### 3. Test organisms

Eisenia fetida andrei Species:

Common name: Earthworm

Source: In-house lab culture; rain of

Age at study initiation: Approximately 7 month old

Feeding during test: Dried cow manure

340 to 350 mg (mean figures Weight at test start:

Maintenance of culture:

Temperature:

Photoperiod: 12 hours light 12 hours dark

Food:

### B. Study design and methods

1. In life dates:

#### 2. Design of biological test

rk vintervals

Shimals for the coposed to By em with this continue of the coposed to By em with Earthworms (Eisenia fetida artarei; approximately 7 month old. & x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed to By 1 02960 SL 200 G; (purity 199.8 g BY1 02960/L = 17.0% w) in an artificial soil system with 10 % peat over a period of 8 weeks. The test item was mixed into the soil. Nomina Pconcentrations were 8.9, 15.8, 28.1, 50.0 and 89.0 mg product/kg dry worght soil, respectively. In addition, a water control was tested. Each jar (plastic boxes; ca. 16.5 cm x 1/2 cm, x 6 cm (length x width x height)) served as one replicate filled with 500 g artificial soil (dry @eight) The depth of the soil layer was approximately 5 cm. The surface area of the soil was 200 cm<sup>2</sup>. The test was conducted at  $20 \pm 2$ °C and 518 to 543 lux at 16 h light and 8 h dark. The artificial soil contained 10% peat, 1% food, 20% kaolinite clay, 68.65% quartz sand and 0.35% calcium carbonate.

## 3. Observation and measurement

Food (dried cattle manuse) was amended weekly dramg the first four weeks. After a period of 4 weeks the adult earth forms were removed from the test vessels and the survivors were counted and their fresh weight was measured. From these data mortality and bromass effects were determined. The test vessels were increated for another 4 weeks under identical climatic conditions. From these the reproduction was determined by counting the number of offspring hatched from the cocoons after this additional test period per test vessel.

#### 4. Statistical analysis

The homogeneity of variances of the data (body weight change and number of surviving juveniles) was checked by Cochean's test. The nomogeneity hypothesis was accepted. The normal distribution of the data was tested by Kothogotov-Smirnov test. The normality hypothesis of the data was accepted.

The data were statistically evaluated by means of a Williams multiple sequential t-test.

The statistical software package ToxRatPro Version 2.09 @ was used for the calculation.



#### RESULTS AND DISCUSSION

### A. Physical and Chemical Parameters

The soil-pH was 6.42 to 6.50. The moisture of the artificial soil was adjusted to nominal 37.5 g water/100 g dry weight artificial soil (nominal 27.3 % soil moisture) corresponding to approximately % of the maximum water holding capacity of the artificial soil. % of the maximum water holding capacity of the artificial soil.

Prior to the start of the test the mean soil moisture was 20.9 %. At day 0 the mean soil moisture 27.0 % and at day 56 it was 25.3 %.

#### **B.** Biological Findings

No mortality of adult earthworms was observed after 28 days of exposure at the test concentrations 28.1, 50.0, and 89mg test item/kg dry weight soil. One worm died during & days in the concentration 15.8mg test item/kg dry weight soil Effects on survival, changeoin body weight and number of juveniles are listed as follows:

Effects of BYI 02960 SL 200 Con mortality, body weight change and reproduction of Table: Eisenia fetida andrei

Nominal concentration of	Mortality after 28 days (%)	Mean body weight change (%) from	Mean number of juveniles after 56	% Mayeniles compared to
test item [mg/kg dry soil]		day 0 to day 28	days of	compared to
control	Q~ %	44.6 ± 809	$2.0 \pm 22.7$	ÿ % -
8.9	<b>* * * * * * * * * *</b>	48.1 ± 8.5 ″	205.3×±39.7 ⊘	93.3
15.8	<i>∞</i> 2.5 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	52. <b>©</b> ± 3.1	190.0@13.5	√9 86.4
28.1	° 0 , 4	9 50×9 ± 75	> 188.8 ± 12.8 * ×	85.8
50		Q43.8 ± 9.1 , C	162.8 ±27.3 * °C	74.0
89	\$ 6° ~	@ 45.4\\4.8 \\	130.0 ±28.8 *4	59.1

<sup>\*</sup> Significant according to Williams Montiple Sequential t-test

### C. Validity Criteria &

The validity criteria of the test according to the adideline were, fulfille

Validity criteria	Recommended	Obtained
Mortality of the adults in the control &	_ ≤ 10/%	0 %
Mean change in growth of the adult of thworks in the control during the exposure period of four weeks	<b>20</b> %	+ 44.6 %
Mean rate of reproduction of juveniles by the control  Coefficient of variance of reproduction in the control  D. Test with toxic reference substance	30 earthworms per control vessel	220 earthworms per control vessel
Coefficient of variance of reproduction in the control of	≤ 30 %	10.3 %
D. Test with toxic reference substance  Reference substance: Date of most recent test: Result:  Defenda fluessig (Carl 15 JAN 2009  Result:  NOEC: 1.25 mg a.i./k		

### D. Test with toxic reference substance

#### E. Biological Endpoints Derived

From the results present	ted above the following biological endpoints can be derived:
4-week figures	
NOEC (growth)	≥ 89 mg product/kg dws
LOEC (growth)	> 89 mg product/kg dry weight soil
8-week figures	
NOEC (reproduction):	8.9 mg product/kg dws
LOEC (reproduction):	15.8 mg product/kg dws
CONCLUSION	ted above the following biological endpoints can be derived:  ≥ 89 mg product/kg dws > 89 mg product/kg dry weight soil  8.9 mg product/kg dws 15.8 mg product/kg dws 15.8 mg product/kg dws  YI 02960 SL 200 G on earthworms ( <i>Eisenia fetida andrei</i> ) can be quantified as mg product/kg dry weight foil.    KIIA 8.9.2/02;
The chronic effect of B	YI 02960 SL 200 G on earthworms (Eisenig Jetida andrei) Pan be quantified as
an overall-NOEC of 8.9	mg product/kg dry weight wil.
Report:	KIIA 8.9.2/02;
Title:	BYI 02960 Difluoroacetic acid. Effects on survival, growth and reproduction on the
	earthworm Eistenia fetida tested in artificial soft with 10% pear
Report No:	LRT-Rg-R-®1710 × × × × × × × × × × × × × × × × × × ×
Document No:	M-39806-01-2
Guidelines:	
D 1.1	OEC 222: 2004
Deviations:	None & & & & & &
GLP:	Les (certified laborators)

The aim of the study was to determine the chronic effects of By I 02960-diffeoroacetic acid (Origin Batch no. BCOO \$984 1; TOX 08889-00; parity 95.8% w/w) ou survive, growth and reproduction of earthworms (Eisen fa fetida andrei).

Adult Eisenia fetida (approx. 5 months ald, 8 x 90 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 10% peat content) to the nominal test concentrations of \$\text{N}\$, 20, \$\text{S}\$, 62 and 110 mg test item/kg dry weight artificial soil. The test item was mix into the soil After 28 days the number of surviving animals and their weight alteration was determined. They were then remove of from the artificial soil. After further 28 days, the number of offspring was determined.

No statistically significant different values for the growth relative to the control were observed at any test concentrations. A cratistically significant different value for the number of juveniles per test vessel relative to the control was beerved only at the bighest test concentration of 110 mg test item/kg dry weight artificial soil.

Overall, based in the brological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is 62 mg test item/kg dry weight artificial soil. Thus the overall LOEC is determined to be 110 mg test item/kg dry weight artificial soil.

#### **MATERIAL AND METHODS**

#### A. Materials

1. Test material

Test item: BYI 02960-difluoroacetic acid (BCS-AA56716)

Type of test material: Substance, technical (pure metabolite)

Chemical state and description: Colourless liquid

Origin Batch number: BCOO 5984-1-1 (Batch code: BCS-AA56216-01-01)

Sample description: TOX 08889-00 Purity: 95.8% w/w

Stability of test compound: Expiry date: 29.07.2010, when store  $\mathbb{Q}$ at  $+25 \pm 5^{\circ}$ C

2. Test solutions

Test item mixed with: Quartz sand

Method of preparation: Test item by s 5 aguartz sand wefe thoroughly mixed into the

artificial soil using a laboratory mixer

Controls: Water control

3. Test organisms

Species: Eisenia fetida andre Common name: Earthworm

Source: In-house lab culture; strain

Weight at test start: 0°0.25 00.45 Maintenance of culture:

Food: Dried cattle manure

B. Study design and methods

1. In life dates March 14, to May 12, 2010

2. Design of biological test

Principles of the testing procedure Adult Cisenia fetida (approx) 5 months old) were exposed in an artificial soil (with 0 % peat content) to BYI 02960-diffuor acetic acid (purity 95.8%) at nominal test concentrations of 11, 26 35, 62 and 100 mentest item/kg downweight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Each eplicate consisted of a jar (plastic loxes; 3. 16.5 cm x 12 cm x 6 cm; length x width x height) filled with 500 g soil. The depth of the soil layer was 5 cm. The surface area of the soil was approximately 300 cm. 10 worms were used per replicate. The test was conducted with 4 replicates per treatment level. In the controls 8 replicates were tested. The test was conducted at  $20 \pm 2$ °C and 579, 524 and 500 Lux at day 0, 28 and 56, respectively at a 16 h light - 8 h dark photoperiod. The artificial soil contained 30% pear, 20% kaolinite clay, 68.65% quartz sand and 0.35% calcium carbonate.

#### 3. Observation and measurements

Food (dried cattle manure) was amended weekly during the first four weeks. After 4 weeks mortality of adult worms, their body weight change and intoxication symptoms were assessed as indicated below in the result section. The test vessels were incubated for another 4 weeks under identical climatic conditions. Then the juveniles were counted.

#### 4. Analytical verification

Not applicable

#### 5. Statistical analysis

The U-test after Bonferroni-Holm (two-sided,  $\alpha = 0.05$ ) was used to determine significant differences of growth between control and treatments.

#### RESULTS AND DISCUSSION

#### A. Physical and Chemical Parameters

Prior to the start of the test the mean soil moisture was 20.2%. At day 0 the mean soil moisture was 27.15% and at day 56 it was 26.7%.

		mg te	storem/kg dry artificial soil	weight	pl	Dvalue
Control	b o .		"O" `			6.46
Difluoroacetic acid			/ 11 <sub>0</sub>	. %		6.37
Difluoroacetic acid			S 20 /			6.43
Difluoroacetic acid		v Ş	″ <u>*</u> \$35		4	6.40
Difluoroacetic acid			62	A.	Ø1	6.39
Difluoroacetic acid			~ 11 <b>.0</b> ~	Ø' ×	ð	6.37

#### B. Biological Findings

Observations on immobilization changes in body weight, number of juveniles and sublethal intoxication symptoms are listed as follows:

Table: Effects of on mortality, body weight change and reproduction of Eisenia fetida andrei

Concentration of test item [mg/kg dry/soil]	%mortality (after 28 days)	% body weight % change after 28 days	number of juveniles/test vessel (after 56 days) mean ± SD	Number of juveniles/surviving adult (after 56 days) mean ± SD
control		66.647.2	$264.8 \pm 30.6$	$26.5 \pm 3.1$
11		61, ®± 3.6	$259.8 \pm 28.2$	$26 \pm 2.8$
20 @	0	653 ± 13.4	$273.5 \pm 27.4$	$27.4 \pm 2.7$
35	<u> </u>	$\sqrt[8]{60.4 \pm 7.0}$	$282.3 \pm 45.8$	$28.2 \pm 4.6$
62		$61.5 \pm 5.5$	$230.3 \pm 22.0$	$23.0 \pm 2.2$
110		$60.1 \pm 3.5$	190.0 ± 32.0*	$19.0 \pm 3.2$

No statistically significant differences to the control for body weight (U-test, Bonferroni-Holm,  $\alpha = 0.05$ ) \* statistically significantly different compared to the control (Williams Multiple Sequential t-test, one-sided smaller  $\alpha = 0.05$ )

No sublethal behavioural changes were observed.

#### C. Validity Criteria

The validity criterion of more than 30 juveniles in each control replicate is fulfilled. The validity criterion of control variation regarding reproduction of less than 30% is fulfilled. The validity criterion of control adult mortality less than 10% is fulfilled.

#### D. Test with toxic reference substance

Reference substance: Derosal fluessig (Carbendazim 360 g/L)

Date of most recent test: January to March 2009

Result: NOEC = 1.25 mg a.i. dry weight so

#### E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

No worm died after 28 days of exposure at the control group. No statistically significant differences for the growth relative to the control were observed at any test concentration. No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 11, 20, 35 and 62 mg test item/kg dry weight apifficial soil.

A statistically significant different value for the number of priveniles per test vessel relative to the control was observed at the highest concentration of 110 metest item/kg by weight artificial soil.

Based on these observations the following are derived

NOEC (mortality):

NOEC (growth):

NOEC (reproduction):

LOEC (reproduction):

A mg/kg dry weight soil

62 mg/kg dry weight soil

60 mg/kg dry weight soil

#### CONCLUSION

The chronic overall NOEC for difluor acetic acid on earthworms (Eisenia fetida andrei) is NOEC of 62 mg/kg dry weight soft.

Report: (2011)
Title: 6-chloronizotinic and (AFOF 161089): Effects on survival, growth and reproduction on
and earth of the Lagrand Tested in a micrai son with 5% pear
Report No: LRT-&g-R-101/11 O
Document No. M 3562-02-2
Guidelines: "   180 11268-2: 1998 (E) \( \sigma^* \) \( \sigma^* \)
OECD 222: April 13, 2004
Deviations: None of O o
GLP. Yes (certified laborator)

#### EXECUTIVESUMMARX

The aim of the study was no determine the chronic effects of 6-chloronicotinic acid (Batch code: AE F161089 00 1B99 0001; parity 98.8% w/w) to earthworms (*Eisenia fetida andrei*).

Earthworms, 8 month old 30 worms used per replicate) were exposed in an artificial soil system with a peat content of 5% over a period of 4 weeks to nominal concentrations of 100 mg/kg (first run; limit test) and 9.5, 16.8, 30.0, 53.4 and 95.0 mg/kg dry weight soil (2nd run), respectively. In addition, a water control was tested.

The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Immobilisation and body weight change in the adults and number of juveniles after another 4 weeks as well as sublethal behavioural effects were used to determine the endpoints.

Overall, based on the biological and statistical significance of the effects observed on growth and reproduction at 100 mg/kg in the first run, it is concluded, that the NOEC for this study is 95.0 mg test item/kg dry weight artificial soil.

#### MATERIAL AND METHODS

#### A. Materials

#### 1. Test material

Test item:

6-chlorenicotinic acid 6-CNA, AE F.161089.

Type of test material:

Substance, technical pure in tabolite.

Chemical state and description: Begge powder

Origin Batch number: 01/265 Batch ode: AE F161089 00 B99 0001)

Material number: AE F161089
CAS#: 532623-8
Purity: 98.8% w/w

Stability of test compound: Expiry date: 09,07.2013, when store to the store of the

#### 2. Test solutions

test item mixed with: Quarte sand

Concentration of vehicle: 5 % peat in 500 g dry weight artificial soil

Controls: Water control

#### <u>3. Test organisms</u>

Species: Serial fetida andrei Common name: Earth form Serial Inzhouse lab culture

Age at study initiation: Symonth Trange less than weeks)

Feeding during lest: Maximum 5 g dry manure moistened with 6mL water once per week

during first 🔊 weeks

Weight at test start: Ringe: 0.23 to 0.44 g per worm

Maintenance of culture:

Temperature:  $\sqrt{22 \pm 2C}$  C

Photoperiod: 12 hours light, 12 hours dark

Food: Wried cattle manure at 14 day intervals

# B. Study design and methods

1. In life dates November 18, 2010 to June 15, 2011

# 2. Design of Mological test

Earthworps (*Eisenia fetida andr*ei; 8 month (range less than 4 weeks)) were exposed to 6-chloronfeotinic acid (code: AE F161089; purity 98.8% w/w) in an artificial soil system over a period of 8 weeks. Concentrations were 100 mg/kg (first run; limit test) and 9.5, 16.8, 30.0, 53.4 and 95 mg/kg dry weight soil, respectively (2<sup>nd</sup> run), mixed into the soil. Due to a statistical significance of the treated

group in the limit test, compared to the control group, a second run was necessary. In addition, a water control was tested. Each replicate consisted of a jar (non-re-usable plastic boxes; ca. 16.5 cm x 12 cm x 6 cm; 1 x w x h) filled with 500 g artificial soil with 5 % peat. The depth of the soil layer was 5 cm. The surface area of the soil was approximately 200 cm<sup>2</sup>. 10 worms were used per replicate. The test was conducted with 4 (8 in the 1st run; limit test) replicates per treatment level. In the controls 8 replicates were tested. The test was conducted at  $20 \pm 2$  °C and 477 - 588 lux at 16 h light. 8 h dark

#### 3. Observation and measurements

After 4 weeks mortality of adult worms, their body weight change and intoxication symptoms were assessed as indicated below in the result section. After removal of the dults the test yessels incubated for another 4 weeks under identical climatic conditions. Then the juveniles were counted

#### 4. Statistical analysis

The homogeneity of variances of the data was checked by Cochran's rest. The normal distribution of the data was tested by Kolmogorov-Smirnov test. The normally distributed data of the limit test were, statistically evaluated by a Pairwise Mann Whitney U-test Procedure, two - soled,  $\alpha = 0.05$ . The data of the dose-response test were statistically evaluated by means of a Williams multiple sequential t-tost, two – sided,  $\alpha = 0.05$ .

#### RESULTS AND DISCUSSION

## A. Physical and Chemical Parameters

The soil-pH was  $6.0 \pm 0.5$ . The water content was 56.8respectively, of the maximum water holding capacity

B. Biological Findings

Observations on immobilization changes in body weight, number fluvences and sublethal intoxication symptom are listed as follows

Effects of AF F161089 on mortality, body veight change and reproduction of Eisenia fetida

Concentration of test	28 drávš	© 28 days	56 days
item <sup>*</sup> Q	% mortality	% body weight change	number of juveniles/test
(mg/kg dry/soil)		(mean ± SD)	vessel (mean $\pm$ SD)
control (1st run)	5 0 Q Q	$72.8 \pm 4.3$	$211.9 \pm 30.2$
\$ <del>0</del> 0		$50.5 * \pm 19.8$	107.4 **± 36.1
control (2nd run)		$59.17 \pm 8.25$	$215.1 \pm 29.2$
<sub>4</sub> < 9.5 < √		$55.82 \pm 10.00$	$208.0 \pm 36.4$
16.8		$56.01 \pm 2.66$	$199.0 \pm 55.3$
30 🔎 🐪		$49.94 \pm 7.45$	$222.3 \pm 34.9$
53.4		$49.52 \pm 6.48$	$180.0 \pm 34.4$
9\$ \( \sqrt{'}		$53.35 \pm 4.43$	$186.0 \pm 16.8$

statistic V significance compared to the control (Pairwise Mann-Whitney U-Test Procedure, two-sided,  $\alpha = 0.05$ )

No sublethal behavioural changes were observed.

<sup>\*\*</sup> statistical significance compared to the control (STUDENT t-test for homogeneous variances, one-sided smaller, α = 0.05) δ

#### C. Validity Criteria

The validity criteria were fulfilled.

Validity criteria	Recommended	Limit-Test	Dose-response- Test
Mortality of the adults in the control	≤ 10%	0	9₽″ ô
Mean change in growth of the adult earthworms in the control during the exposure period of four weeks	should not exceed - 20 %	72.8%	59.2%
Rate of reproduction of juveniles (no of earthworms per control vessel)	≥ 30	Ž11.9	215.4
Coefficient of variance of reproduction in the control	≤ 30% 🔻	Q 14.2	V \$3.6 V

#### D. Test with toxic reference substance

per control vessel)		_ 50 (	9			23.4	
Coefficient of variance of reproduction is	n the control	≤ 30%	,	Q 14.2	. 0	\$3.6 ₩	
D. Test with toxic reference substa	nce Carbendazim JAN to MAR						
Reference substance:	Carbendazim	ı SC 360∘G	i (Derøsal	flüssig) _ @	~ ~ <u>`</u>		
Date of most recent test:	JAN to MAR	201		4 . ~		4	
	A.		Ü Ç		, Ő		¥°
Test object			Ô		a fetida		
Reference test item		"	Control	O Carb	endazim SC	360 G	
mg t.i./kg dry weight artificial soil			Q ~	1,25	2.50	5.0	
Mortality of adult earthworms after 28		*\\\'\'\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	0 冷	<b>80</b>	D' Ç	J 0	
Body weight change of adults from day	0 to day 28 🛭	<u>6</u> ]	+ 44.6	J 955.4 €	+34.2	¥ + 32.2 <b>*</b>	
Standard Deviation			£8.9 (	± 4.6°	≈± 6.6%,	± 2.8	
Statistical comparison to the control		- C	~~	,	n.s.O	S.	
Mean number of offspring/test vessel	ter 56 days (	Ž O	220.0	247.5°×	15795 **	20.8 **	
Standard Deviation	Ø		±Q2.7	± 243	≈©± 5.4	± 15.6	
Statistical comparison to the control		\(\frac{1}{2}\)	V 🖇	øn.s. ⋄	(S) S.	S.	

- Result of a Williams multiple sequential t-test two-sided,  $\alpha = 0.05$
- \*\*
- Result of a Williams faultiple sequential t-test, one-sided smaller,  $\alpha=0.05$  mean value statistically significantly different compared to the control (p=0.05) S.
- mean value not statistically significantly different compared to the control ( $p \ge 0.05$ ) n.s.

the test was sensitive to the reference test The results of the refe item.

# E. Biological Endpoints Derived

From the results presented wove the following biological endpoints can be derived:

96 mg/kg dry weight soil NOEC (growth): NOEC (reproduction) 95 mg/kg dry weight soil LOE (Growth) 100 kg/kg dry weight soil LOẼ (reproduction) 100 mg/k@dry weight soil

The chronic effect of 6-coloronic tinic acid (AE F161089) on earthworms (Eisenia fetida andrei) can be quantified as an overall-NOEC of 95 mg/kg dry weight soil.



#### **IIA 8.10** Effects on soil microbial activity

#### **IIA 8.10.1** Nitrogen transformation

Report:	KIIA 8.10.1/01;	(2009)	
Title:	BYI 02960 a.s.: Determination	of effects on nitrogen trans	Firmation in soil 6
Report No:	FRM-N-130/09		
Document No:	M-359803-01-2	A	
Guidelines:	OECD guideline 216, 2000		
Deviations:	None		
GLP:	Yes (certified laboratory)		

#### **EXECUTIVE SUMMARY**

The aim of the study was to determine the effects of BY 02960 (Origin Batch No; 2009-000239; Batch code: BYI 02960-01-03; TOX 08508-00; purity 96.2% w/wito the witrogen turnover of soil microflora.

Rates of 0.3 and 3.0 mg/kg a.i./ha (corresponding to 0.4 and 4.0 mg a.i. kg dro weight soil) were applied on loamy sand soil. After the ancendment with Lucerne-grass green meal the nitrogen turnover was measured at day 0, and after 7, 140 and 28 days of incubation

The deviation from the control did not exseed 25% after 28 nitrogen turnover of soil microflora.

#### MATERIAL AND METHOD'S

#### A. Materials

#### 1. Test material

Test item:

Type of test material.

Chemical state and description: Substance, technical

Beige powder

2009-000239 (Batch code BYI 02960-01/203)

Sample description:

2(5H) Guranone, 4-[[60-chloro-3-pyridinyl)methyl](2,2-CAS name:

difluoroethod)amino?

CAS#:

4-[(6-chloropyr)din-3-ylmeth (2,2-difluoroethyl)amino]furan-2(5H)-one

#### 2. Test solutions

Method of preparation:

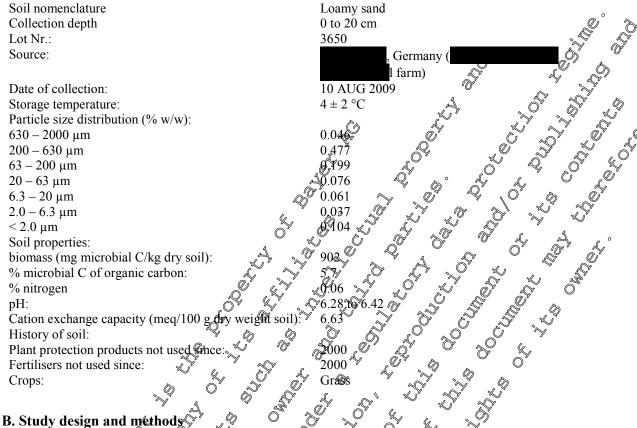
Controls:

Water control

Jamino]furan-2(5H)-on

Water control

#### 3. Test soil



1. In life dates

## 2. Design of biological test

Rates of 0.3 and 3.0 mg/kg a Pha (corresponding to 0.4 and 4.0 mg a.i. kg dry weight soil) were applied on logmy sand. After the amendment with Lucenne-grass-green meal (consisting of 40.6% Ctotal, 0.05% C, 2.5% N) the nitrogen turn over was measured a day Q and after 7, 14 and 28 days of incubation. In addition a water control was rested. Each replicate consisted of a jar (brown glass bottles; 0.5 L) filled with 300 g dry weight test soll. The test was conducted with 3 replicates per treatment level. The test was conducted at 20

## 3. Observation and measurement

At day 0, and after 7, 14 and 28 days of incubation subsamples [moist samples (equivalent to 10 g dry weight)] were taken from each jar. The content of mmonium, nitrite and nitrate was measured with a Bran Lübbe Autoanalyzec

All calculations were performed using Microsoft Excel 2003.

The percentage difference in the quantities of nitrate-N formed between control soils and treated soils were expressed as absolute values and determined as follows:

((treatment rates – control rates)/control rates) x 100 % = % difference.

Rateš wero expressed in "mg nitrate-N/kg dry weight soil/day".

Homogeneity of variances was determined by Cochran's Test,  $\alpha = 0.05$ . Depending on the results the appropriate T-tests were performed. In the T-test, the values of nitrate-N/kg dry weight soil/time

interval/day from control soils and treated soils were compared. The statistical calculations were carried out using ToxRatPro 2.09 (2002).

#### RESULTS AND DISCUSSION

#### A. Physical and Chemical Parameters

The soil-pH was 7.19. The water content was 40 to 50% of the maximum water holding capacity before the start of the study.

## **B.** Biological Findings

During the 28-day test, 0.4 mg BYI 02960 a.i./kg dry weight soil cause a temporary timulation of the daily nitrate rates at the time interval 0-7 days after treatment in a logary sand soil amended with Luzerne-grass-green meal. At the end of the test (1428 day interval), differences in the nitrate-N rates between control soil samples and treated soil samples are <25 % and meet the trigger values of above mentioned guideline for a termination of the study.

Table: Effects of BYI 02960 on nitrogen turnover of the soil inicroflora indoamy sand (transformation per time interval and day).

Time	BY 02960 a.s. 4 5 5 5	~
Interval	mg nitrogem (N)/kg dys/time/interval/day (mean ± SD)	
(days)	(mean ± SID)	
	-	
	Control 4.0 mg/kg dry weight soil 4.0 mg/kg dry weight soil	
	Nitrate-N difference Nitrate-N difference to control	nce
	to control to control	trol
0-7	$0.45$ $\pm$ $0.13$ $0.6$ $\pm$ $0.37$ $3$ $n.s.$ $0.56$ $\pm$ $0.22$ $24$ $n.s.$	S.
7-14	3.52	ķ
14-28	$1.75$ $\pm$ $0.03$ $0.71$ $\pm$ $0.05$ $0.05$ $0.04$ $0.04$ $0.04$ $0.05$	

<sup>\* =</sup> Statistically significant difference to the control (Stadent D Test, two-sides,  $\alpha = 0.05$ ).

#### C. Validity Criteria

The validity criterion of control variation of these than 15% is fulfilled.

## D. Test with toxic reference substance

A reference test with softum choride Conducted 2009 demonstrated that 16 g NaCl/kg dry weight soil had distinct and long-term (28 days) influence of microbial mineralization of nitrogen.

#### CONCLUSION

BYI 02960 has negligible effects on pitrogen turnover of soil microflora when applied at 300 g and 3 kg at hectare.

Report:	(2011)
Title:	6-chloronicotinic acid (AE F161089): Determination of effects on nitrogen
	transformation in soil
~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	FRM-N-156/11
	M-408028-01-2
	OECD guideline 216, 2000
Deviations:	None
GLP:	Yes (certified laboratory)

n.s. = No statistically significant difference to the control (Student-t Test, two-sided,  $\alpha = 0.05$ ).

#### **EXECUTIVE SUMMARY**

The aim of the study was to determine the effects of 6-chloronicotinic acid (Batch code: AE F161089

A rate of 1 kg test item/ha (corresponding to 1.33 mg test item /kg dry weight soil) was applied on silty sand. After the amendment of Lucerne-grass-green most the sit. sand. After the amendment of Lucerne-grass-green meal the nitrogen turnover was measured at day and after 7, 14 and 28 days of incubation.

The deviation from the control did not exceed 25% after 28 days. 6-chloropicotinic acid (AE) has negligible effects on nitrogen turnover of soil microflora.

#### MATERIAL AND METHODS

#### A. Materials

### 1. Test material

Sithy sand

#### 3. Test soil

Soil nomenclature	Silturand
	Silty sand
Collection depth	0 to 20 cm
Lot Nr.:	F2.30110
Source:	Germany/
Date of collection:	05 JAN 2011
Storage temperature:	4 ± 2 °C
Particle size distribution (% w/w):	
63 – 2000 μm	0.62
$2-63~\mu \text{m}$	
< 2.0 μm	0.62 to 0.28 t
Soil properties:	
% organic carbon:	
Biomass (mg microbial C/kg dry soil):	23 1
% of soil organic carbon content:	Ø.78 × × × × × ×
Mineralized nitrogen (mg/100g dry weight soil)	23 h
Cation exchange capacity (meq/100 g dry weight soil)	Silty sand 0 to 20 cm F2.30110  Germany/ 05 JAN 2011 4 ± 2 °C  0.62\$ 0.28\$ 0.988  231 277 12.4  9006 Fallow land since 2006 Fallow land since 2006
pH:	
Cation exchange capacity (meq/100 g droweight soil):	¥12.4°
The state of the s	2006 P P P P P P P P P P P P P P P P P P
History of soil:	
Plant protection products not used since:	
Fertilisers not used since:	2006
Crops:	Fallow land since 2006 V
	4 2 4 4 4

## B. Study design and methods

1. In life dates

March 1500 April 21 3911

### 2. Design of biological test

A rate of 1 kg test item ha (corresponding to 1.33 and test item /tog dry weight soil) was applied on silty sand soil. After the amendment of Lucertic-grass-green meal (consisting of 40.6% Ctotal, 0.05% Cinorg, 25% N) the nitrogen ternover was measured at day 0, and after 7, 14 and 28 days of incubation. In addition, a water control was tested, Each replicate consisted of a jar (brown glass bottles; 0.5 L) filled with 300 g de weight test foil. The test was conducted with 3 replicates per treatment level. The test was conducted at  $20 \pm 2^{\circ}$ 

#### 3. Observation and measurements.

At day 0, and after 7, 14 and 28 days of incubation subsamples (moist samples (equivalent to 10 g dry weight)) were taken from each jar. The content of ammonium, nitrite and nitrate was measured with a Bran Lübbe Autoanalyze 3.

#### 4. Statistical analysis

All calculations were performed using Migrosoft Excel 2003.

The percentage differences in the quantities of nitrate-N formed between control soils and treated soils were expressed as absolute values and determined as follows:

((treatment rates – control rates)/control rates) x 100 % = % difference.

Rates were expressed in "mg nitrate-N/kg dry weight soil/day".

Homogeneity of variances was determined by Cochran's Test,  $\alpha = 0.05$ . Depending on the results the appropriate T-tests were performed. In the T-test, the values of nitrate-N/kg dry weight soil/time

interval/day from control soils and treated soils were compared. The statistical calculations were carried out using ToxRatPro 2.10 (2010).

#### RESULTS AND DISCUSSION

#### A. Physical and Chemical Parameters

The soil-pH was 7.08 to 7.13. The water content was 41.94 to 44.58% of the maximum water folding capacity.

#### **B.** Biological Findings

The deviation from the control was not significant at any time interval and did not exceed 25% after 28 days.

Table: Effects of AE F161089 on nitrogen turnover of the soil microflora in silty sord given as deviation from the control

		O Application rate of o
Time		4 6-chipronic acid
Interval	control	1.33 mg/kg dry weight soft
(days)	Nitrate-N <sup>1)</sup>	Wrate-NIV W Maifference to control &
0-7	$-1.75 \pm 0.15$	-1/28 × 0.04 × 57 n.s.
7-14	$2.03 \pm 0.09$	$  (9)   \approx 0.00$
14-28	$0.88 \pm 0.16$	Q 0.89 ± Q.03

<sup>1)</sup> Rate: Nitrate N mg/kg dry weight wil/time interval/day

#### C. Validity Criteria

The validity criterion of control variation of less that 15% is fullithed

### D. Test with toxic reference substance

A reference test with Sodium chloride conducted in 2011 showed that 16 NaCl/kg dry weight soil had distinct and ong-term (> 28 days) influences on microbial principality ation of nitrogen.

#### CONCLUSION

### IIA 8.10.2 Carbon mineralization

Report:	KIIA \$10.2/01; (2011)
Title:	BYL02960 a.s.: Effects on the activity of soil microflora (Carbon transformation
	test) test)
Report No:	AA, 10 48,058 C Q X
Document No:	M-417√94-01-2 0
Guidelines:	OF D guideline 237, 2000
Deviations:	None 🗸 🧠
GLP:	Yes (certified Daboratory)

#### EXECUTIVECHUMNARY

The sim of the study was to determine the effects of BYI 02960 (Origin Batch No: 2009-000239; Batch code: BY 02960-01-03; TOX 08508-01; purity 96.2% w/w) to the carbon turnover of soil microflora. Rates of 0.3 and 3 kg a.i./ha (corresponding to 0.4 and 4.0 mg a.i./kg soil dry weight) were applied on sandy loam (USDA nomenclature). After the amendment of 2000 mg glucose/kg dry weight to soil

n.s. = No statistically significant difference to the control (Student-t Test, two sided,  $\alpha^{\square}$  0.050

subsamples at day 0, and after 7, 14 and 28 days of incubation the carbon turnover was measured during a period of at least 12 hours.

The deviation from the control did not exceed 25% after 28 days.

BYI 02960 has negligible effects on carbon turnover of soil microflora when applied at 0.3 and 30kg

#### MATERIAL AND METHODS

#### A. Materials

#### 1. Test material

BYI 02960 Test item: Type of test material: Substance, technical Beige powder Chemical state and description:

Batch number: 2009-000239 (Batch code:

Sample description:

CAS name:

difuoroethyl)amino

CAS#: 95@59-40-8

IUPAC name: AM (6-chloropya)din

Purity: 96.2% w/w@

Expiry date. Stability of test compound:

#### 2. Test solutions

Test item mixed with:

was treated with other 100 ground quartz sand/kg Šieved soil (2 mm) Method of preparation;

Controls:

## 3. Test soil

Soil nomen lature Sandy loam OUSD A Gromen chature) logny sand DIN nomenalature)

Collection depth 096 20 Km

Lot Nr.: Ã0603 °C

Source: Date of collection:

Storage temperature:

Particle size distribution (% v

Sand 50 2000 μm Silt (2 – 50 μm) Clay (2 μm)

Soil properties: % organic carbon.

% humus: Biomass (mg microbial C/kg dry soll): % microbi@ C of ganic Carbon 2.38 % nitrogen 0.13

6.4 History of soil

Plant protection products not used since: 1991 2003 Fertilisers not used since: Crops: Fallow land

#### B. Study design and methods

1. In life dates August 26 to September 23, 2011

#### 2. Design of biological test

Rates of 0.3 and 3 kg a.i./ha (corresponding to 0.4 and 4.0 mg a.i./kg soil dry weight) were applied on sandy loam (USDA nomenclature) /loamy sand (DIN nomenclature) soil. After the amendment of 2000 mg glucose/kg dry weight to soil subsamples at day 0, and after 7, 14 and 28 days of incubation the carbon turnover was measured during a period of at least 12 hours. In addition a water control was tested. Each replicate consisted of a jar (stainless steel vessels; 4 L) filled with 550 g div weight test soil. The test was conducted with 3 replicates per treatment level. The dest was conducted at 19.6 to 22.0°C.

#### 3. Observation and measurements

At day 0, and after 7, 14 and 28 days of incubation subsamples (moist samples; equivalent to 100 g dry weight) were amended with 2000 mg glucose/kg dry weight. The carbon-droxide production was measured with a respirometer (BSB digi SELUTEC) over a period of at least 02 hours.

#### 4. Statistical analysis

The cumulative O2 consumption was calculated using regression analysis over 15 hours

The coefficients of determination were greater than 0.90 in all cepticates and on all days of measurement.

Standard deviation and coefficient of variation west calculated.

The percent deviation (D) from control was calculated for each sampling date by the following equation:

 $C_1 = O_2$  released in the treated group in  $O_2$  kg soil@d.w./h

C= O2 release in the control group in mg 0/2/kg soil de h

Statistical evaluation of the test sesults 2-sided Student-t-test for homogeneous variances at 5 % significance level) was performed.

#### RESULTS AND DISCUSSION

### A. Physical and Chemical Parameters

The soil-pH was 6.2 to 6.3 The water content was 46.63 to 49.26 of the maximum water holding capacity.

#### B. Biological Findings

No adverse effects of BYI 02960 on carbon transformation in soil were observed at either test concentrations (040 mg/kg dry soil) after 28 days. Only negligible deviations from control of 40.1 % (test concentration 0.40 mg/kg dry soil) and -0.9 % (test concentration 4.00 mg/kg dry soil) were measured at the end of the 28-day incubation period.

Table: Effects on carbon transformation in soil after treatment with BYI 02960 a.i.

Days after application	Control	0.40 mg test item/k equivalent to 0.3		4.00 mg test iten weigh equivalent to 3 kg	t W
	O <sub>2</sub> consumption [mg/kg soil d.w./h]	O <sub>2</sub> consumption [mg/kg soil d.w./h]	Deviation from control [%] <sup>1</sup>	O <sub>2</sub> consumption [mg/kg soil d.w./h]	Devotion from control
0	12.54	12.51	-0.2	△12.26*s	5° -2° .4
7	11.27	11.28	÷0.1	11.27	<b>≈</b> 0.0 ≈
14	10.61	10.95	3.2	© 10.34 °	×-2.5, Ø
28	9.64	9.66	+0.1	9.56 °	J -0,8V

The calculations were performed with unrounded values.

Variation in control replicates on carbon turnover of the soil wicroflow in sandy loan Table: (USDA nomenclature) given al % c.v@

	day 0	Ş	Yay 7	y LO	day 14		day 28
% c.v.	0.7	Q	(% 0.7°		× 1.75	Q"	2.2 O

The deviations can be regarded as negligible

### C. Validity Criteria

The validity criterion of control variation of less than 15% is fulfilled

### D. Test with toxic reference substance

A reference test with Pinoterbeconducted 05, JAN 2011 to 02 FEB 2011 revealed inhibitions of 27.8, 45.5 and 47.8% at 68, 16 and 27 mg/

micoflora when applied at up to 3 kg a.i./hectare.

#### Rates of recovery following treatment **IIA 8.10.3**

Studies on recovery following treatment are not required as EVI 02960 is not intended for use in products for soil sterilisation and has no long term effects on soil micro-organisms

# Effects on marine and estuarine organisms

For the European registration of an active substance inclusion of an active tests on marine and estuarine organisms are not a data recorrement. However, acute and chronic studies have been conducted as requirements for registration in USA and Canada.

The data from marine fish Cypridodon variegatus), the shrimp Americamysis bahia and the oyster are summarized in the following subchapters.

<sup>1)</sup> based on  $O_2$  consumption; - = inhibition; + = stimulation

<sup>\*</sup>s statistically significantly different to control (Student test for homogoneous ariances



#### **IIA 8.11.1** Marine or estuarine organisms acute toxicity LC50/EC50

Report:	KIIA 8.11.1/01; , C.S. & , C.V. (2009)
Title:	Acute Toxicity of BYI 02960 Technical to the Sheepshead Minnow (Cyprinodo)
	variegatus) Under Static Conditions
Report No:	EBRVP034
Document No:	M-357479-01-1
Guidelines:	OECD Test Guideline 203:
	EPA-FIFRA § 72-3
	OPPTS 850.1075
<b>Deviations:</b>	None \(\varphi\) \(\varphi\)
GLP:	Yes (certified laboratory)
	Some data (screening for contaminants in water) was not performed according to
	GLP, as described in the study report

#### **EXECUTIVE SUMMARY**

The aim of the study was to determine the acute effects of B 02960 (Origin Batch No. 2009) Batch code: BYI 02960-01-03; TOX 08508-00; purity 26.29 to sleepshead minnow (Cyprinodon variegatus).

Cyprinodon variegatus (10 fish per reatment level) were exposed in a static system over a period of 96 hours to nominal concentrations of 5,00, 10,0,20.0,40.0 and 80.0 mg a in (corresponding to analytically verified concentrations of 5%, 10.4, 21.0, 40.4 and 83 Ping a j./L; 10 to 15% of nominal). In addition, a water control and solvent control were tested,

Mortality and sublethal behavioural effects were used to determine the endpoints. Based on analytical NOEC was determined to be 83.9 mg a.i./L the findings the biological endpoints are reported as mean measured figures ...

The 96-hour-LC<sub>50</sub> was > 83.9 mg  $\approx 1.7$ highest concentration tested.

# MATERIAL AND MOTHODS

#### A. Material

#### 1. Test material

Test item: Sabstance, technical Type of test material

description:
Origin Batch number:
Sample docale. Beige powder

2009-000239 (Batch code BYI 02960-01-03)

Sample description:

CAS name (5H)-Furance, 4-[[&chloro-3-pyridinyl)methyl](2,2-difluoroethyl)amino]

CAS#;

IUPAC name: 44(6-chloopyricho 3-ylmethyl)(2,2-difluoroethyl)amino]furan-2(5H)-one

Purity:

Expire date: 16.01.2011, when stored at  $+25 \pm 5$ °C Stability of test empound: Water solubility Approximately 80 mg a.i./L under test conditions

Dimethylformamide (DMF)

Concentration of vehicle: 0.1 mL/L

Method of preparation: Sonicated for 20 minutes Control Water control and solvent control

Evidence of undissolved None

material:

#### 3. Test organisms

Species: Cyprinodon variegatus

Species: Cyprinodon variegatus

Common name: Sheepshead minnow

ABS073008

Source:

Feeding during test: None

Length at test start: 25.2 ± 1.6 mm (range: 22.5 to 29.0 mm)

Weight at test start: 0.24 ± 0.05 g (0.17 to 0.33 g)

Static loading: 0.08 g fish/L

Maintenance of culture:

Temperature: 22 ± 1.0 °C

Photoperiod: 16/8 hour light/dark photoperiod

Food: Tetramin flake food and brine shrimp

Period of maintenance prior to study initiation:

Mortality during acclimatisation period:

B. Study design and methods

1. In life dates April 20 to 24, 2009

2. Design of biological test

Cyprinodon variegatus were exposed to BYI 02960; Qurity 96.2% In a static system over a period of 96 hours. Nominal concentrations were 5.00, 10.0, 20.0, 40.0 and 80.0 mg a.i./b. In addition a water 96 hours. Nominal concentrations were 5.00 10.0 Q0.0, 40.0 and 80.0 mg a.i./. In addition a water control and solvent control were tested. Each vessel (glass aquaria; 38 L (495 x 25 4 x 30.5 cm)) filled with 30 L synthetic sea water served as one replicate 40 fislower cused per replicate. Toxicology Laboratory consists of artificial sea calts (HW Machiemix Professional from Sei) mixed with blended soft water to produce a salinity of approximately 17 parts per mousand (%) Length of fish at test start was  $25.2 \pm 1.6$  mg/ (range: 22.5 to 29.0 mm). Body weight of fish at test start was  $0.24 \pm 0.05$  g (0.17 to 0.33 g). The staric big original loading was 0.08 g fron/L. The test was conducted with one replicate per treatment level.

#### 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at day 0 and 4 from all concentrations. LCMS/MS was used as analytical method. The limit of quantification (LOQ) was 0.3 mg a.i./L. The range of linearity was 0.001 to 09 mg/L?

Mortality of fish, intoxication symptoms and physical-chemical water parameters were assessed as indicated below in the result section.

5. Statistical analysis

Not applicable



#### **RESULTS AND DISCUSSION**

#### A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test solutions are summarized as follows:

Test temperature: 21.8 to 22.6 °C (mean: 22.1 °C)

pH: 8.0 to 8.2
Dissolved oxygen (mg/L): 6.6 to 7.7 mg/L
Dissolved oxygen (% saturation): 83 to 97 %

Photoperiod: 16 hours light / 8 hours dark Light source Cool white fluorescents

Light/dark transition period: 30 minutes
Light intensity: 745 to 1016 lux
Salinity:  $17 \pm 1 \%$ 

#### **B.** Analytical Findings

Analytical verification of test solutions revealed measured concentrations of 36, 10.4, 21.0, 40.4 and 83.9 mg a.i./L; 101 to 112 % of nominal calculated as arithmetic mean. Biglogical results are reported as mean measured. Detailed analytical results are presented in the following table:

Table: Nominal and measured concentrations of BYI 02960

Nominal Test Concentration (mg a.i./L)	Day O Measured (mg a,iGL)	New) > Company	Day 4 Measured (rag a.i./L)	γ ·	Mean Measured (mg-s.i./L)	Mean S D	Mean % Nominal
Control	<0.30	NA NA	~ < 0.30°	NA <sub>c</sub>	<b>₹</b> 0.30	NA	NA
Solvent Control	₹9.30	√ NA	]	y Na	& <0.30 <sub>y</sub>	NA	NA
5	© 5.6	\$113 <b>Q</b>	Ĵ\$.6 ∑	111	© 5. <b>%</b>	0.04	112
10	\$ 10 <del>8</del> 3	10.7	10,20	\$102 <u>↓</u>	<b>2</b> 0.4	0.34	104
20	, 20.9	, §102	y 21(j)	≈ 106¢	<b>21</b>	0.17	105
40	<b>240</b> ○	0100 & O	<b>49</b> .8 2	y 102	40.4	0.51	101
80	® 83.4 S	104,	×84.5 \$	1 <del>0</del> 6 ≪	83.9	0.82	105

## C. Biological Findings

Mortality was observed as listed below

Table: ©Effect of BY 102960 on mortality of Cyprinodon variegatus

Exposure time	24	h S	48	h	72	2 h	96	h
Measured test concentration (mg/a/i./L)	no of	A dead	dead %	% dead	no. of dead	% dead	no. of dead	% dead
Control		$\mathcal{L}_0$ $\mathbb{Q}$	QC	0	0	0	0	0
solvent control		Ø 0,S	<b>@</b>	0	0	0	0	0
5.6	~0 &	0	<b>@</b> 0	0	0	0	0	0
10.4		<b>~</b> 0 '	$\circ$ 0	0	0	0	0	0
21.0	0	0 0	0	0	0	0	0	0
40.4		S 0	0	0	0	0	0	0
83.9.		0	0	0	0	0	0	0

No sublethal behavioural changes were observed.

#### D. Validity Criteria

The validity criterion of control mortality less than 10% was fulfilled. The validity criterion of oxygen saturation above 60% is fulfilled.

#### E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

96-hour-figures:

LCso: > 83.9 mg a.i./L
lowest concentration with effect (LOEC): > 83.0 mg a.i./L
highest concentration with no effect (NOEC): 83.0 mg a.i./L
highest concentration with no mortality (NOLEC): mg a.i./L

#### **CONCLUSION**

The acute effect of BYI 02960 on sheepsheat minnow (Cyprinodon variegatus) can be quantified as a 96-hour-LC<sub>50</sub> of > 83.9 mg a.i./L, the highest concentration tested.

Report:	KIIA 8, 1.1/02; 5, S.P., 7, Z. & H.O. (2009)
Title:	BYI 02960: A 96-Hour Shell Deposition Test with the Pastern Syster (Crassostrea
	virginica) 2 0 2
Report No:	EBRVP023
Document No:	M-36166-01-12 Q Q
Guidelines:	OPPTS 850.1025
<b>Deviations:</b>	None O S O S O S
GLP:	Yes (certified laboratory)  Periodic screening of saltwater for potential contaminants was not performed
	Reriodic screening of saltwater for potential contaminants was not performed
GLP:	Ounder GLP V V V V

#### EXECUTIVE SUMMARY

The aim of the study was to determine the acute effects of By 1 02960 (Origin Batch No: 2009-000239; Batch code: BYI 02960 01-03, TOX 0850800; purity 96.2% www) on shell deposition of the Eastern Oyster (*Crassostrea Virginica*).

Oysters (mean valve height of 35.1 ± 27 mm; range 30.2 to 40.1 mm, 20 per treatment level) were exposed in a flow through system over a period of 96 hours to nominal concentrations of 0.94, 1.9, 3.8, 7.5, 15 and 30 mg a.i./L (corresponding to analytically verified concentrations of 0.90, 1.8, 3.6, 7.3, 15 and 29 mg a.i./L; 95 to 97% of cominal). In addition a saltwater control was tested. The test was conducted at concentrations near the functional limit of solubility of BYI 02960 in a saltwater system. Shell deposition, mortality and sublethal behavioural effects were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as mean measured figures. There were no mortalities of clinical signs of to active observed at any concentration tested.

The 96-how-EC was determined to be 29 mg 3.i./L, the 96-hour-NOEC was determined to be 29 mg a.i./L.

#### MATERIAL AND METHODS

#### A. Materials

#### 1. Test material

Test item: BYI 02960 Type of test material: Substance, technical Beige powder Chemical state and description:

2009-000239 (Batch code: BYI 02960-01-034) Origin Batch number:

Sample description: TOX 08508-00

CAS name: 2(5H)-Furanone, 4-[[(6-chboro-3-pyriding/methyl](2

difluoroethyl)amino]

951659-40-8 CAS#:

IUPAC name: 4-[(6-chloropyridin=

Purity: 96.2% w/w

Stability of test compound: Expiry date:

#### 2. Test solutions

Dimetrylformamide (L Vehicle:

0.10mL/L 👟 Concentration of vehicle: Mixed at least 20 times Method of preparation: Saltwator control Controls:

Evidence of undissolved material: N

#### 3. Test organisms

Species: Common name: Eastern oyster

Source:

Examination and proparation of

oysters:

ylmethyl)(2,2-difluoroethyl)(mino)furan-2(\$PI)-Oysters showed no evidence of spawning of parasitation. Prior to testing, 3 to 5 mm of the new peripheral shall growth of each oyster was removed by grinding the shell to a blunt core using a fine-grit grinding wheel

Size at study@nitiation: mean valve height of  $35.1 \pm 2.7$  mm/mange: 30.2 to 40.1 mm)

Concentrated volumes of algal suspension (approximately 2.9 to 5.8 10^9 Feeding daring test:

per wster per day) supplied with a peristaltic pump

Maintenance of cultu

Temperature:

Food:

machie michalgae (Reed Mariculture, Campbell, CA)

## B. Study design and methods

1. In life dates

Crassostrea virginica (mean valve height of  $0.5.1 \pm 2.7$  mm; range: 30.2 to 40.1 mm) were exposed to BYI 02960 (purity 96.2%) in a flow through system over a period of 96 hours. Nominal concentrations were 0.94, 19, 3.87.5, 19 and 30 mg a.i./L. In addition a saltwater control and a solvent control were tested. Each replicate consisted of one vessel (glass aquaria; 54 L) filled with 27 L (depth of water: 15.6 cm) natural seawater filtered and diluted in order to adjust salinity to 20%. 20 oysters were used per replicate. Osters showed no evidence of spawning or parasitation. Prior to testing, 3 to 5 mm of the new peripheral shell growth of each oyster was removed by grinding the shell to a blunt edge using. The test was conducted with one replicate per treatment level.

#### 3. Method of analytical verification

For analytical verification of the test item concentrations samples were taken at 0, 48 and 96 hours from all concentrations. BYI 02960 (purity: 99.4%) served as analytical standard. High-performance liquid chromatography (HPLC) was used as analytical method. The limit of quantification (LOQ) was 1,400 mg a.i./L.

#### 4. Observation and measurements

Oysters were inspected visually at approximately 6, 24, 48, 72 and 96 hours after test initiation to determine the numbers of mortalities and the numbers of individuals exploiting sub-lectual signs of toxicity. At the end of the test, the longest finger of new shell growth on each oyster was measured to the nearest 0.1 mm using calipers. Shell deposition, mortality and indexication symptoms of the cyster as well as physical-chemical water parameters were assessed as indicated below in the result section.

#### 5. Statistical analysis

There were no statistically significant differences (p. 0.05) between the negative and solvent control groups, using an appropriate t-test. Therefore, the control data were project for comparisons among the BYI 02960 treatment groups. Shell growth inhibition was calculated for each treatment group as the percent reduction in mean shell growth relative to the mean control shell growth. The stell deposition data were evaluated for normality and homogeneity of variance using the Chi-Square test and Levene's test, respectively. Since the data grassed the assumptions of normality and homogeneity the data in the treatment groups were compared to the pooled control data using analysis of variance (ANOVA) and Bonferroni's t-test to identify any significant differences.

## RESULTS AND DISCUSSION

### A. Physical and Chemical Parameters

Measurements of physical and chemical parameters of the test solutions are summarized as follows:

Test temperature
pH:
Dissolved oxogen (mg/L):
Photoperiod:
Light source
Light intensity:
Salinity:

19.2 to 21.2 °C
8.1 to 8.2
6.940 7.4 mg/L
6.940 7.4 mg/L
7.4 mg/L
8.6 % of saturation
16 hours light and 8 hours darkness
Fluorescent onlbs
30 min
788 lux at water surface
20%

## B. Analytical Findings 🕼

Analytical verification of test solutions revealed measured concentrations of 0.90, 1.8, 3.6, 7.3, 15 and 29 mg/a.i./L, respectively 395 to 37% of nominal) calculated as arithmetic mean.

Detailed analytical results are presented in the following table:

Table: Nominal and measured concentrations of BYI 02960

	Day 0 (1	New)	Day	2	Day	4	Mean	0
Nominal Concentration (mg a.i./L)	Measured (mg a.i./L)	Percent Nominal	Measured (mg a.i./L)	Percent Nominal	Measured (mg a.i./L)	Percent Nominal	Measured (mg a.i./L)	M@n Percent Sominal
Control	< LOQ#		< LOQ		< LOQ	Ž	(ک	
solvent control	< LOQ		< LOQ		< LOQ	Ø,		
0.94	0.894	95.1	0.911	96.9	0.888	<b>△</b> 94.5	0.8	<b>\$96</b>
1.9	1.83	96.1	1.81	95.5	1.78	<b>№</b> 93.8	18	y 95 y
3.8	3.61	94.9	3.65	96.	3.56	93.6	©3.6	9 <b>%</b>
7.5	7.32	97.6	7.3	97.4	7.28	97.1	<b>Q</b> 7.3	, <b>3</b> 7
15	15.0	99.9	14.8	<b>99</b> .0	14.9	99.3	<sup>™</sup> 15%	
30	29.1	97.0	29.4	₽98.1	29%3 ∂	° 97. <b>5</b>	<b>42</b> 9	U 97.Ç

 $<sup>^{\#}</sup>LOQ = 0.4 \text{ mg a.i/L}$ 

### C. Biological Findings

Observations on mortality, signs of toxicity and mean shell deposition and growth inhibition are summarised below:

There were no mortalities among oysters in any treatment or control group during the lest

All oysters appeared normal throughout the 96-hour exposure or riod

Table: Effect of BYL02960 on shell deposition of Crassostrea virginica

Mean measured concentration	Shell Deposition <sup>A</sup> (mean ± SD) (mga)	Mean percent reduction
Negative Control	3.2 1.4	\$ 4- Q
Solvent Control	2.6 ± 1.4	
Pooled Control	0 02.9 ±4.4 ×	D 3
0.9	2.8±1.1	
√J.8	2	© ≥ ©£.9
3.6 Q	\$3 ± 1.6	√y 0 <sup>y</sup> -13
7.3	2.5 ±3.1 ~	13
15	y 3.5 ≠ 1.4 ° °	-19
29	25/± 1, 15/	13

A Mean and standard deposition for 20 overters.

There was no significant difference in shell deposition between the treatment groups and the controls.

#### D. Validity Criteria

The validity criterion of control mortality less than 10% is fulfilled. The validity criterion of control shell growth > Imm is fulfilled. The validity criterion of oxygen saturation above 60% is fulfilled.

#### E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

Based on mean measured concentrations

29 mg a.i./L

96-hour 10 50 for Shell Deposition:

> 29 mg a.i./L

B No significant difference from pooled control using Bonferroni t-test (p > 0.05)

#### **CONCLUSION**

The acute effect of (BYI 02960) on Crassostrea virginica can be quantified as a 96-hour-EC<sub>50</sub> of > 29 mg a.i./L. The NOEC was determined to be 29 mg a.i./L.

The acute effect of (	(BYI 02960) on <i>Crassostrea virginica</i> can be quantified as a 96-hour-EC <sub>50</sub> of
	NOEC was determined to be 29 mg a.i./L.
Report:	KIIA 8.11.1/03; , S.P., , T.Z. & , H.O. (2009)
Title:	BYI 02960: A 96-Hour Static Acute Toxicity Test with the Saltwater Mysic
	(Americamysis bahia)
Report No:	149A-236 V Q Q Q X
Document No:	M-364620-01-1
<b>Guidelines:</b>	EPA OPP 72-3(b)
	EPA OPPTS 850.1035
<b>Deviations:</b>	None S S S S S S S S S S S S S S S S S S S
GLP:	Yes (certified laboratory)
	Screening of saltwater for contaminants was not performed under GLP, as detailed
	in the study report A A A A A A A A A A A A A A A A A A A

EXECUTIVE SUMMARY

The aim of the study was to determine the acute effects of BYI 02960 (Sample description: TOX 08508-00 (Batch ID: 2009-900239) purity 96.2% w/w & Americany as baloa.

Juvenile Americamysis bahia \$24 hours old 20 per treatment level) were exposed in estatic system over a period of 96 hours to nominal concentrations of 0.13, 0.22, 0.360 and 1.0 and a.i./L, (corresponding to analytically verified concentrations of 0.12, 0.21, 0.35, 0.58 and 0.98 mg a.i./L, respectively).

In addition a water control was tested. Two replicate test chambers were maintained in each treatment and control group, with W saltwater mysids in each test chamber, for a total of 20 mysids per test concentration.

Mortality and sublethal behaviour effects were determined by visual interpretation. Based on analytical fundings the biological endpoints are reported as measured figures.

The 96-hour-EC  $_{50}$  was 0.26 ring a.i./L (95% confidence limits: 0.12 - 0.58 mg a.i./L), the 96-hour-NOEC was determined to be 0.120 ing a

#### A. Materials

1			∽ " 1	
		OCT.	matorial	
		COL	111144671141	

By 1 02960

Substance, tectuncal
Beige powder
102900022313
Batch number
2009-000239
Sample description
CAS name
Purity:
Stability:

2(5H)-Furanone, 4-[[(6-chloro-3-pyridinyl)methyl](2,2-

4-[(6-chloropyridin-3-ylmethyl)(2,2-difluoroethyl)amino]furan-2(5H)-one

Expiry date: 16.01.2011, when stored at  $\pm 25 \pm 5$ °C

#### 2. Test solutions

Vehicle: None

The stock solution was stirred with a wire whisk for approximately 1-2 minutes and sonicated for approximately 35 minutes Method of preparation:

minutes and sonicated for approximately 35 minutes.

Controls: Water control

Evidence of undissolved material: None

#### 3. Test organisms

Species: Americamysis bahia Common name: Mysid shrimp

Source:

Age at study initiation: < 24 hours old

Live brine shrimp rauplii (Artemia sp.) Feeding during test:

Maintenance of culture:

Temperature:

22.0 to 27.0°G

16 hours of light and 8 hours of darkness

Live bring shrimp flauplii. Artemia and 1 Photoperiod: Food:

#### B. Study design and methods

1. In life dates

#### 2. Design of biological test

Americamysis bahia (< 24 hours old) were prosed to BYI 02960 purity 96.2%) in a static system over a period of 96 hours. Nominal concentrations were 0.13, 0.22, 0.36, 0.60 and 7.0 mg/a.i./L. In addition a water control was tested. Each tessel (glass baker 2 L, filled with 1.5 L'test Sution) served as one replicate. 10 mysids were used per replicate. The test was conducted with two replicates per treatment level. Saltwater mystas were impartially assigned to exposure chambers at test initiation. Observations of mortality and other signs of toxicity were made at 18, 24, 48, 72 and 96 hours (± 1 hour) after test initiation. The comulative percent mortality observed in the treatment groups was used to determine LC50 values@at 24, 48, 72 and 96, hours. 4

#### verification

For analytical verification of the test item concentration samples were taken at 0, 48 and 96 hours from all concentrations XYI 02960 (purity: 29.4%) Served as analytical standard. High-performance liquid ascanalytical method. The limit of quantification (LOQ) was 0.05 mg a.i./L.

#### 4. Observation and measurement

chemical water parameters were assessed as indicated Mortality, intoxication symptoms below in the result section

#### 5. Statistical analysis

The probit method was used to calculate the 24-hour LC<sub>50</sub> and the 95% confidence interval. Nonlinear interpolation was used to calculate the 48, 72 and 96-hour LC<sub>50</sub> values and binominal probability was used to calculate the 95% compdence intervals. Due to the method used to calculate the 96-hour LC<sub>50</sub> value the slope of the dose response curve could not be calculated. The no-mortality concentration and NOBC were determined by visual interpretation of the mortality and observation data.



#### RESULTS AND DISCUSSION

#### A. Physical and Chemical Parameters

/leasureme	nts of physica	al and che	mical parame	eters of the	test solution	ns are sum	marized as fo	ollows	
							<b>*</b>		)* <sup>V</sup>
Test temper	ature:		25 ± 2 °C (	range: 23.2	2 to 26.2 °C)	L T	Ž.		
рН:			8.1 to 8.2			.1	Q		à
Dissolved oxygen (mg/L):			6.5 to 7.4 r	ng/L			2		4
Dissolved o	xygen (% satu	ration):	> 89%	,	Ø	@\Y			. W
Photoperiod	1:		16 hours of	f light and	hours of dar	kness	.0 3		S
Light source	e		fluorescent	light bylbs	; <u> </u>	<i>,</i>			1
Light/dark t	ransition perio	od:	30 min		Q"	Ö° 1	4	O L	
Light intens	sity:		798 lux	Q)"		, Ø*	\$ \O`	``````````````````````````````````````	
Salinity:			20 ‰	(1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	' <i>5</i> ° 4				
			_				* v		
				)'@`		~0"	0° 1,	1	
) Analytia	ol Findings		<b>△</b>						
3. Analytic	al Findings					A.S			
<b>3. Analytic</b> Analytical v	eal Findings verification of	f test solut	ions réveale	) V	d conventration	Ons of 0.12	,	0.58 and	
<b>3. Analytic</b> Analytical v .98 mg a.i.	ral Findings verification of /L calculated	f test solut as arithm	ions revealed	hmeasured	l conventration	Ons of 0.12	2, \$21, 0\$5, San massure	0.58 and	
<b>3. Analytic</b> Analytical v  1.98 mg a.i. Detailed ana	eal Findings Verification of Verification of	f test solut as arithm	25 ± 2 °C ( 8.1 to 8.2 6.5 to 7.4 r > 89% 16 hours of fluorescent 30 min 798 lux 20 %  ions revealed etic mean Britanied in the fi	hmeasured ological r	l concentration	ons of 0.12	2. § 21, 0.35, san measure	0.58 and	
Analytical values of the second secon	eal Findings verification of /L calculated alytical result	f test solut as arithm s are prese	ions revealed etic mean Bi	measured cologgical r	l conventration	ons of 0.12	2, <b>9</b> 21, 035, sean measure	0.58 and	
Analytical value of the Analytical value of the Analytical value of the Analytical of the Analytical of the Analytical of the of the Analytical of the Analy	eal Findings verification of /L calculated alytical result Nominal	f test solut as arithm as are preso	ented in the formation	ollowing thions of By	able: 5		2, \$21, 0.35, \$2, \$2, \$3, \$4, \$5, \$6, \$6, \$6, \$6, \$6, \$6, \$6, \$6, \$6, \$6	0.58 and	
Detailed and able: Nominal	Nominal  Day	and meason	ented in the formation	ollowing to	able: S YI 02960 S Day		Mean	0.58 and	
Detailed and Table:  Nominal Conc.	Nominal  Day  Measured	and meason  O  Peocent	ented in the formation	ollowing to tions of By Percent	able: YI 02960 Day Measured 2	4 Percent	Mean Measured	Mean Percent	
Detailed and Table:  Nominal Conc. (mg a.i/L)	Nominal  Day	s are presonand meason  O  Peogrent Nominal	ented in the formation	ollowing to the trions of By Percent Nominal	able: S YI 02960 S Day	4 Percent	Mean	Mean	
Detailed and Cable:  Nominal Conc. (mg a.i/L) Control	Nominal  Day  Measured (mg a.i./L)	s are present Nominal	ented in the formed concentral was a line of the concentral was a line of	collowing to the collow	able: VI 02960 VDay Measured (mg a.i./L)	Percent Nonairal	Mean Measured ong a.i./L)	Mean Percent Nominal	
Nominal Conc. (mg a.i/L) Control 0.13	Nominal  Day  Measured (mg a.i./L)  - 4  0.128	and meason 0 Percent Nominal 98.3%	mied in the formed concentral was a line of the concentral was a line of t	tions of B'  2 C  Percent Nominal	Able: Day  Measured  (mg.a.i./L)  0.123	Percent Nominal	Mean Measured ong a.i./L)	Mean Percent Nominal - 92%	
Nominal Conc. (mg a.i/L) Control 0.13 0.22	Nominal  Day  Measured (mg a.i./L)  -  0.128	and meason o Procent Nominal 98.3%	med concentral  Measured (mg a.i./L),  0.122	collowing to the collow	Able:	Percent Nontinal  y	Mean Measured Ong a.i./L) - 0.12 0.21	Mean Percent Nominal - 92% 95%	
Nominal Conc. (mg a.i/L) Control 0.13 0.22 0.36	Nominal  Day  Measured (mg a.i./L)  -  0.128  0.214  0.372	s are prese and meason 0 Percent Nominal 98.3%	Measured (mg ari./L), 0.122 0.347	Percent Nominal 95.0% 2 96.4%	Able: (**)  (**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(**)  **(*	Percent Nontinal	Mean Measured ong a.i./L)  -  0.12  0.21  0.35	Mean Percent Nominal - 92% 95% 97%	
Nominal Conc. (mg a.i/L) Control 0.13 0.22	Nominal  Day  Measured (mg a.i./L)  -  0.128	and meason 0 Procent Nominal 98.3%	Measured (mg a.i./L).  0.122  0.347  0.573	collowing to the collow	Able:	Percent Nontinal  y	Mean Measured Ong a.i./L) - 0.12 0.21	Mean Percent Nominal - 92% 95%	

### C. Biological Findings

The single mortality of the 6.12 mca.i./L peatment group was a mysid that was missing and assumed to be dead at the 72-hour observation period, this was considered to be incidental to treatment and was therefore was excluded from the LC Calculations.

Signs of toxicity (loss of equilibrium and that the very noted in the 0.21, 0.35, 0.58 and 0.98 mg a.i./L treatment groups, but all surviving mysids were normal in appearance and behavior at test termination.

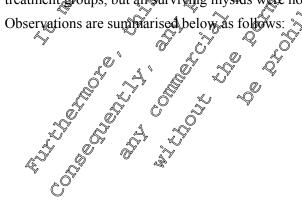


Table: Effect of BYI 02960 on mortality of Americamysis bahia

Mean	Cum		Observation period						0
meas.	ulativ	24	24 hours 48 hours 72 l		nours	96 1	nours		
(mg a.i./L)	e % mort.	No. Dead	Obs.	No. Dead	Obs.	No. Dead	Obs.	No. Dead	Obs.
control	0	0	10 AN	0	10 AN	0		O.C.	t©AN (
Control	- "	0	10 AN	0	10 AN	0 8	10 AN	~0°	20 AN
0.12	5	0	10 AN	0	10 AN	1	9 AN		9 A
		0	10 AN	0	10 AN	0,Q	10 AN	0	10 AN
0.21	60	0	10 AN	0	€ 10 AN	Q.	8 AN	<b>3</b> Q,	ØAN Ø
		0	10 AN	2 🚑	8 AN	\$6 <sub>0</sub>	°3AN/VN	<b>L</b> 9	$O_1$ AN
0.35	50	2	8 AN	3 2	7 AN /	y 7, 0°	3 AN N	0,8 \$	2 A®
		0	8 AN, 2N	<i>Q</i> ′	10 AN		DO AND	2,0	& XN
0.58	100	8	2 C	ďØ.	0°-,V	40	~ ~	"10 <sup>"</sup>	4 -
		8	2 C	10		©10 C	ř <u>-</u>	O10	{^,
0.98	100	10	- %	J 10%	~ ×	104		10 🖓	
_		10	- 2	-10%	0,7 - 4,	10/	`\ - \\	10	2 <u>7</u>

Observations: AN = appear normal; N = log of equilibrium C

D. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived, based on mean measured concentrations:

48-Hour LC<sub>50</sub>:
72-Hour LC<sub>50</sub>:
96-Hour LC<sub>50</sub>:
96-Hour LC<sub>50</sub>:
95% Confidence Limits:
No-Mortality Concentration:
No-Observed-Effect Concentration:
0.12 mg 41./L
0.12 mg a.i./L

CONCLUSION

The source of the content and the

The acute effect of BYL 2960 on Americannysis balta (for perly Mysidopsis bahia) can be quantified as a 96-hour-Lesso of 626 mg/a.i./L+95 % confidence limits: 0.12 - 0.58 mg a.i./L).

The following chronic test is on mysid shripps is also included in this section as there is no specific dossign point for the chronic text is on mysid shrimps is abso in dossign point for the chronic texticity on marine species.

Report:	KIIA 8.11.1/04; , M.B., , T.Z. & , H.O. (2011)
Title:	BYI 02960: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid
	(Americamysis bahia)
Report No:	EBRVP038
Document No:	M-420783-01-1
Guidelines:	OPPTS Number 850.1350: Mysid Chronic Toxicity Test SASTM Standard E 1191-03a: Standard Guide for Conducting Life-Cycle
	Toxicity Tests with Saltwater Mysid
<b>Deviations:</b>	None & S
GLP:	Yes (certified laboratory)

#### **EXECUTIVE SUMMARY**

The aim of the study was to determine the chronic effects of BYI 02060 (Sample description: TOX 08508-01 (Batch ID: 2009-000239); purity 96.0%) or the survival reproduction and growth of the mysid shrimp (Americamysis bahia)

Fifteen neonates (<24 h old) of Americannysis bahia per repricate were exposed in a flow-through system over a period of 28 days to reminal concentrations of 46, 8.0, 13.9, 4.2 and 42 µg a.i./L (corresponding to analytically verified concentrations of 4.2, 7.8, 13 2, 23.6 and 40 ug a, L; (91 to 98% of nominal). In addition a water control and solvent control were tested. On Day 14 of the test, ant and c appared mysid and signs of toxicity alternative used to determine the production and growth were used to determine the production and growth a after mysids attained sexual maturity, male and female adults were paired in each treatment and control

#### 2. Test solutions

Vehicle: Dimethylformamide (DMF)

Concentration of vehicle: 0.1 mL/L

Controls: Water control and solvent control

Natural seawater filtered and diluted with fresh water to 20 % and UV-sterilized Water media type:

3. Test organisms

Americamysis bahia (formerly Mysidersis bahia) Species:

Common name: Mysid shrimp Source: Inhouse-culture Age at study initiation: < 24 hours old

Feeding during test: Live brine shrimp (Artemia salina)

hydration) wice daily

Maintenance of culture:

Temperature:

Photoperiod:

Live bring shrimp trauplit inriched with Atgama 3050 Mysids also periodically supplemented with Skeletonema Food:

### B. Study design and methods

1. In life dates:

#### 2. Design of biological te

Less than 24 hours old be onates of Americanysis behia were exposed to BYI 02960 (purity 96.2 %) in a flow-through system over a period of 28 days. Nominal concentrations were 4.6, 8.0, 13.9, 24.2 and 42 μg a.i./L. In addition a water control and solvent control were dested flow through was achieved with a continuous flow diluter mixing 25 μ stock solution per minute into 250 μL dilution water per minute. Mysids were separated in getention chambers uptil they could be sexed and separated pairwise into the pairing chambers. The test was conducted with 4 replicates per treatment level. During the first 14 days the juveniles were kept in 9 L glass aquaffa containing 2.5 L test solution. In these tanks the juveniles of each replicate were enclosed in 2 Leompartments with two nylon mesh covered holes on opposite sides. The flow tate was 18 volume changes per day

During the last 4 days of the test adult mysids were selected for pairing. Each pair was kept in 10 cm diameter petri dishes with sides of nylon mesh screen. The petri dishes were placed in 19 L glass aquaria containing 17.5 Otest solution. The flow rate was 5 volume changes per day.

For analytical verification of the dest item concentrations samples were taken at days -2, 0, 6, 14, 15, 21 and 28, respectively, from all concentrations BYI 02960 (purity 99.4%) served as analytical standard. HPLC was used as analytical method. The limit of quantification (LOQ) was 2.00 µg a.i./L.

### 4. Observation and measurements

Immobilisation grow and reproduction of mysids and physical-chemical water parameters were assessed as indicated below in the result section.



#### 5. Statistical analysis

Survival data was considered to be discrete-variable data, while reproduction and growth data were considered continuous-variable data. Discrete-variable data were analyzed using Chi-square and Fisher's Exact tests to identify treatment groups that showed a statistically significant difference from Shapiro-Wilk's test and for homogeneity of variance using Levene's test (p = 0.01). The data for all parameters passed the assumptions of normality and 11 the pooled control (p < 0.05). All continuous-variable data were evaluated for normality using the parameters passed the assumptions of normality and homogeneity of variance. Those treatment recans that were significantly different from the pooled control means were identified using Donnett stest (p < 0.05). All statistical tests were performed using a personal computer with SAS

#### RESULTS AND DISCUSSION

#### A. Physical and Chemical Parameters

Measurements of physical and chemical parameters

Test temperature:

pH: Dissolved oxygen (mg/L):

Dissolved oxygen (% saturation):

Photoperiod:

Light/dark transition period: Salinity:

# B. Analytical Findings

solutions are summarized as follows:

range

outs light/10 hours dark
ninutes
11%

arithmetic mean. Biological
uted in the following 1 Analytical verification of test solution revealed measured concentrations of 4.2, 9.8, 13.2, 23.6 and 40 μg a.i./L (91 to 98 % of nominal) calculate@as arithmetic mean. Biological results are reported as mean measured. Detailed analytical results are presented in the following table:

Nomital and measured concentrations of N 10290 Table:

	Measured Configuration (ug a i /I )							
Nominal Concentration (µg a.i./L)	S day 0	alay 6 %		©ay 15.	day 21	day 28	Mean	Percent Nominal (%)
control		S < LOO	<i>uayı</i> ⊕4	S LOQ	·	< LOQ	Mican	(70)
solvent control	LOQ	< LQQ	O LOQ O	< L000	< LOQ	< LOQ		
4.6	0 3,50	4.5	¥ 4.4	24.3	4.5	4.4	4.2	91
8.04	7.9	<b>9</b> 8.0 🚓	708		7.9	8.0	7.8	98
1209	° Ø2.2 ° Ø	14.3	<b>2</b> 2.6 %	13.1	13.3	13.7	13.2	95
24.2	22.8	23/6	\$\times 23.9\times \times	22.7	24.4	24.0	23.6	98
42	≪y 37,85°	~ <b>4</b> 2.4 <sub>C</sub>	40.2	39.1	41.7	41.4	40.0	95

# C. Biological Findings

Observations on mortality from the and reproduction are listed as follows:

Table: Effect of BYI 02960 on mortality and reproduction of Americamysis bahia

Mean Measured Concentration (µg a.i./L)	% juvenile survival to pairing on day 14	% adult survival to test termination on day 28	mean number of young of produced per reproductive day
control	100	83.0	$\sim 0.396 \pm 0.119$
solvent control	95.0	92.5	$\sqrt{9} 0.450 \pm 0.167$
pooled control	97.5	88.0	$0.423 \pm 0.137$
4.2	85.0	86.1	0.358 0.250
7.8	93.3	92.3	0.267± 0.145
13.2	78.3*1	84.6 <b>V</b>	$0.230 \pm 0.90$
23.6	95.0	88.2	0. 436 ± 0.D57**
40.0	93.3	81.3	

<sup>\*</sup> Statistically significant decrease in survival in comparison to the pooled control using Figher's Exact test (p < 0.05).

"Statistically significant decrease in reproduction in comparison to the pooled control using Dunnert's test (p < 0.05).

While the decrease in survival was statistically significant in comparison to the pooled control of was for considered to be treatment-related since the difference was slight and was not dose responsive.

#### Table: Effect of BYI 02960 on growth of Americanysis balia

Mean Measured	Length at d	ay 28 (mm) 🛴 🦠	Body weight	day <b>28</b> (mg)
Concentration	Ó		Mode &	Female
(μg a.i./L)	Male 👋	Female V	Morfe 👸	
control	$8.15 \pm 0.244$	8 <b>9</b> ± 0.29 P	0) 1.02 ± 0.100	1.38 \(\frac{1}{2}\) 0.204
solvent control	8.14 ± 0.469	$99.97 \pm 0.392$	$1000 \pm 0.102$	$5 \pm 0.091$
pooled control	8.15 🕊 🕽 . 195	8.28 ± 0.257	4.01 ± <b>6</b> 097	$\Psi$ .27 ± 0.191
4.2	$7.85 \pm 0.297$	8.25 0.203	0.928 70.096	
7.8	$7.89 \pm 0.268$	8,33 ± 0.163	♦ 0.889 ± 0.105	$1.24 \pm 0.137$
13.2	$8.00 \pm 0.363$	\$32 ± QQ62	v Ø.943 ± 0.1121 ≥	v 1.24 ± 0.086
23.6	8.01 ± 9.217 <sub>4</sub> / 3	8.16 ± 9.214 ×	Ø.917 <b>≰</b> 0.043 🌂	$1.13 \pm 0.150$
40.0	8.16 ± 0.03	© 8.34 €0.110 €	0.923@0.045	$1.21 \pm 0.060$

#### D. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived

28 day-figures:

NOEC (adult survival);

NOEC (reproduction)

NOEC (growth in terms of total length and weight)

LOEC (lowest observed effect concentration)

13.2 µg a.i./I

highest concentration with no effect (overall NOEC).

13.2 µg a.i./I

13.2 µg a.i./I

#### CONCLUSION

Reproduction, measured as mean number of young produced per reproductive day, was the most sensitive biological endpoint reasured. Therefore, the chronic effect of (BYI 02960) on *Americamysis bahia* as the highest concentration with the effects on mortality, growth and reproduction can be set to 13.2 µg a.i./L. as the measured decrease in survival at 13.2 µg a.i./L, although found to be statistically significant in comparison to the pooled control, should not be considered treatment-related since the difference was slightly and was not dose-responsive

#### **IIA 8.11.2** Marine/Estuarine fish - salinity challenge

This is not an EC data hence; data/documents were not created and are not submitted.

#### **IIA 8.12** Effects on terrestrial vascular plants

IIA 8.12 Ef	fects on terrestrial vascular plants
The summary of th	is study is presented below, as it is a core requirement. The test has been performed
with the lead formu	ılation BYI 02960 SL 200G.
Report:	KIIA 8.12/01; H., 2010 D.
Title:	BYI 02960 SL 200 g/L – Effects on the vegetative vigour of eleven species of non-
	target terrestrial plants (Tier 1)
Report No:	VV10/002
Document No:	M-397734-01-2
Guidelines:	OPPTS 850.4150 (1996);
	OECD Guideline 227 (2006)
<b>Deviations:</b>	None A O O O
GLP:	Yes (certified laboratory)

#### **EXECUTIVE SUMMARY**

The purpose of this specific study is to evoluate the potential sobe effects of SYI 02960 SL 200 g/L (Sample description: TOX0885400; Barch ID: 2009 001255; Material No.: 797 18845; Specification No.: 102000021884-01) on the vegetative vigour of elever non-target terrestrial plant species following a post-emergence 410 g a.i. ha application of the product onto the folding of plants

A total of eleven species were fested in this vegetative vigour test including seven dicotyledonous and four monocotyledong species representing nine plant families,

At the 2-4 leaf stage, plants (except Allium copy, which was treated at the \$2 leaf stage) were sprayed once with BYI \$2960 \$200 g/L at an application rate of 410 g and a volume rate of 200 L/ha.

Each pot (replicate) contained 4 plants and there were 30 plants treated (i.e. 8 replicates). Control pots were treated with de-ionized water.

Following application pots were grown and maintained under glasshouse conditions. Survival of the treated plants and visual phytotoxicity were recorded 7,914 and 21 days after application and assessments were made agains the water treated controls. The study was terminated 21 days after

Following a foliar application of BYI 02960 SL 200 L applied at 410 g a.i./ha (corresponding to 2.4 kg product/ha) to eleven terrestrial non-target point species, no adverse effects on survival, visual phytotoxicity, growth, shoot ength and shoot dry weight above 25% effect were observed in this and she in a graph of the control of vegetative vigour study. Only marimal responses were observed, typically within the range of natural variability.

#### MATERIAL AND METHODS

#### A. Materials

1. Test material

BYI 02960 SL 200 G Test item:

Formulated product (soluble (liquid) concentrate) Type:

Chemical state and description: Clear brown liquid 102000021884-01 Specification No.: Material number: 79718845 Sample description: TOX 08854-00 2009-001253 Batch No .: BYI 02960: 200 A Nominal content of active substance:

L according to certificate of BYI 02960: 1720% w/w, 199.8Q Analytical content of active substance:

analysis

Expiry date: 20.03.2010, when stored at 25 ± 5°C in original container in the back (also accorded to the stored to Stability of test compound:

container in the dark (also acceptable from

Density:

2. Vehicle and/or positive control

Negative control: Positive control: 3. Test organisms

Source

Test units:

Photoperiod:

Temperature. Humidity

Climatic conditions:

Species (Common name):

Brassica spapus (pilseed fape) Cucum sativa (cucumber) Fagasyrum esculentum (buckwheats)

Glyeine mo (soybean) Lagctuca Sativa (Settuce)

Lycopersicon Sculentum (tomato)

Monocotyledonae: *Alliyum cepiq* (onion) Ă√ena s@iva (oat) Lolium perenno (ryegrass

Zea mays (corn) Different, mostly commercial, sources, see report, page 18

Commercial plastic flowed pots (13 cm diameter), 8 pots per treatment group Cach prepared with 4 plants

Glasshouse conditions

At lost 16 hours light; natural daylight supplemented by artificial

8°@day, 1 ± 8°C night

±3€0%

Irrigation by bottom watering via saucers standing below each pot Watering: Details of Nutrient Medium Equid Excilizer was added in the saucers on test day 7 for all tested species, Cucumis sativus was fertilized also on days 1 and 14.

February 25 to March 31, 2010

A total of given plant species were tested in this vegetative vigour test including seven dicotyledonous and four monocotyledonous species representing nine plant families.

The following species were treated: Beta vulgaris (sugar beet), Brassica napus (oilseed rape), Cucumis sativus (cucumber), Fagopyrum esculentum (buckwheat), Glycine max (soybean), Lactuca sativa

(lettuce), Lycopersicon esculentum (tomato), Allium cepa (onion), Avena sativa (oat), Lolium perenne (ryegrass) and Zea mays (corn).

Each pot (replicate) contained 4 plants and there were 32 plants treated i.e. 8 replicates. Control pos were treated with de-ionized water.

The spray solution was applied once, at test initiation on the leaves and above-ground portions plants. The blank control spray solution was 200 L deionized water/ha. The test item was dissol deionized water and was applied once with 200 L/ha using a spray chambet equipped with an or nozzle, with nozzle height set at 30 cm above the target area (highest leaf density).

Following application, pots were maintained under glasshouse conditions with a temperature contri at 23  $\pm$  8°C during day and 18  $\pm$  8°C at night with a 16 h photoperiod.

The blank control was 200 L deionized water/ha. Plants were exposed to the

#### 3. Observation and measurements:

The parameters measured were survival, visual phytotoxical, plant growth stage, shoolengt and shoot dry weight. Observations were recorded 7, 1/4 made against the water treated controls

#### 4. Statistical analysis

Statistical analysis of data was performed to obtain significant for shoot length and shoot dry weight effects, carried out using the Parwise Mann Whitney-U-Test (one maller;  $p \le 0.05$ ) by ToxRat statistics.

### RESULTS AND DISCUSSIO

### A. Environmental Parapaeters

Pots were kept in glasshouses under conditions as described above (see material and methods).

## B. Biological Findings

Analysis of BYI 02960 of the tested application rate revealed it to be 99% of nominal.

As a result of a foliar application of BYI 02960 SL 200 g/L with \$10 g a.i./ha to eleven plant species, this study revealed avery low lever of phytotoxicity. There were no adverse effects on surviving with any of the species tested

There were limited phytotoxic symptoms in this study with slight chlorosis, necrosis and stunting in oilseed rape cucumber, buckwheet, tomato and corn,

Buckwheat (Fagopyrum esculentum) was the most sensitive species for shoot dry weight, with 12% reduction, which was statistically significant.

A summary of the findings from a single application of 410 g a.i./ha to eleven plant species tested is summarised in the following table:

Species	Survival (% inhibition)	Phytotoxicity	Shoot Length (% reduction)	Shoot Dry Weight (% inhibition)		
	Dicotyledoneae					
Beta vulgaris	0	0	-2.3	6.5		
Brassica napus	0	0 - 10%	-1.0	7.0		
Cucumis sativus	0	10 - 20%	-2.1	4.5		
Fagopyrum esculentum	0	10%	3.1	12/.0		
Glycine max	0	0	0.6	Q11.1 <sub>Q</sub>		
Lactuca sativa	0	0	-4.70	, O 0,70° ,		
Lycopersicon esculentum	0	10 - 20%	\$.1	10 10 S		
	Moi	nocotyledoneae	Q			
Allium cepa	0	_AQ <sub>/</sub>	© <sup>₩</sup> -2.8 ×	9.3 \$		
Avena sativa	0	, ©	2.1	-1.8		
Lolium perenne	0	Øy 0 /	4.2 Q	3.0		
Zea mays	0	♥0 - 10% @	0.3	<b>A.1</b>		

Bold figures for shoot dry weight are statistically significant (Pairwise Mann-Whitney-U-jest, one-sided smaller; p < 0.05)

### C. Validity Criteria

This study can be considered valid as the validity criterion of at least 90% survivateduring the study period was achieved for the untreated controls of all species.

### CONCLUSION

Following a foliar application of BXV02960 SL 200 g/L applied at 410 g a.i./ha (corresponding to 2.4 kg product/ha) to elever terrestrial non-targer plant species, no adverse effects on survival, visual phytotoxicity, growth, shoot length and shoot dry weight above 25% effect were observed in this vegetative vigour study. Only minimal responses were observed, typically within the range of natural variability.

Report: 🔊	KIIA 8,92/02;4 91., 2000
Title:	BYI 02960 SI 200 g/L – Effects on the seedling emergence and growth of eleven
	spectes of non-target terrestral plants (Tier, 19
Report No:	SE10/001
Document No:	( <sub>4</sub> M-397 <b>72</b> )7-01- <b>2</b> ,7
Guidelines:	OPCD Cuidding (98 (2008)
	OEOD Guideline 208 (2006)
Deviations:	None None
GLP:	Yes (certified faboratory)

# EXECUTIVE SUMMARX

The purpose of this specific study is to evaluate the potential phytotoxic effects of BYI 02960 SL 200 g/L (Sample description: TQX 08854-00; Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 402000921884-01) on the seedling emergence and growth of eleven non-target terrestrial plant species following a pre-emergence application of the product onto the soil surface at a rate of 440 g a pha.

A total of eleven species were tested in this seedling emergence and growth test including seven dicotyled flows and four monocotyledonous species representing nine plant families.

Five seeds of each species were sown in pots in the glasshouse. The soil surface of the pots was sprayed with BYI 02960 SL 200 g/L applied at 410 g a.i./ha and a volume rate of 200 L/ha. Each pot (replicate)

contained 5 seeds and there were 40 seeds treated (i.e. 8 replicates). Control pots were treated with deionized water.

Following application, pots were grown and maintained under glasshouse conditions. Emergence, survival of the emerged seedlings and visual phytotoxicity were recorded 7, 14 and 21 days after application and assessment were made against the water treated controls. The study was terminated 21 days after application.

Following a soil surface application of BYI 02960 SL 200 g/L applied at 410 g a.i./ha (corresponding to 2.4 kg product/ha) to alcount to the second surface application of BYI 02960 SL 200 g/L applied at 410 g a.i./ha (corresponding to 2.4 kg product/ha) to alcount to the second surface application of BYI 02960 SL 200 g/L applied at 410 g a.i./ha (corresponding to 2.4 kg product/ha) to alcount to the second surface application of BYI 02960 SL 200 g/L applied at 410 g a.i./ha (corresponding to 2.4 kg product/ha) to alcount to the second surface application of BYI 02960 SL 200 g/L applied at 410 g a.i./ha (corresponding to 2.4 kg product/ha) to alcount to the second surface application of BYI 02960 SL 200 g/L applied at 410 g a.i./ha (corresponding to 2.4 kg product/ha) to alcount to the second surface application of BYI 02960 SL 200 g/L applied at 410 g a.i./ha (corresponding to 2.4 kg product/ha) to alcount to the second surface applied at 410 g a.i./ha (corresponding to 2.4 kg product/ha) to alcount to 2.4 kg product/ha (corresponding to 2.4 kg product/ha) to alcount to 2.4 kg product/ha (corresponding to 2.4 kg product/ha) to alcount to 2.4 kg product/ha (corresponding to 2.4 kg product/ha) to alcount to 2.4 kg product/ha (corresponding to 2.4 kg product/ha) to alcount to 2.4 kg product/ha (corresponding BYI 02960 SV 200 G
Formulated product (soluble (liquid) concentrated)
Clear brown liquid
102000021884-01
'97 (3845
'07 08864-00
'09-091253
'YI 02960: 270 9/L
'R02960: 77.0% J/w, 199.8 g/L 2cciysis
'4 g/nL
rividate: 20.03.20
'iner.in.00 2.4 kg product/ha) to eleven terrestrial non-target plant species, no adverse effects on emergence, seedling survival, visual phytotoxicity, growth, shoot length and shoot bry weight above 25% effective were observed in this seedling emergence and growth fudy. Only minimal responses were observed typically within the range of natural variability.

### MATERIAL AND METHODS

### A. Materials

### 1. Test material

Test item:

Type:

Chemical state and description: Specification No.:

Material number: Sample description: Batch No .:

Nominal content of active ingresient:

De-ionized where None container in the date (also acceptable from +2 to +30°C)

### 3. Test organisms

Species (Common name): Dicotyledonae:

Beta vulgaris (sugar beet) Brassica napus (oilseed rape) Cucumis sativa (cucumber)

Fagopyrum esculentum (buckwheat)

*Glycine max* (soybean) Lactuca sativa (lettuce)

Lycopersicon esculentum (tomato

Monocotyledonae:

Allium cepa (onion) Avena sativa (oat) Lolium pereine (ryegrass)

Zea mays (Corn)

Different, mostly commercial, sources, see report page 18 Source: Commercial plastic flower pots (10.5 cm/diameter), 8 pots per Test units:

treatment group, each prepared with Seeds "

Climatic conditions: Glasshouse conditions

Af Jeast 16 hours light; natural day! Photoperiod:

Temperature:

Humidity:

Watering:

### B. Study design and methods

February 25 1. In life dates:

### 2. Experimental treatments

A total of eleven plant species were tested in this seeding energence and growth test including seven dicotyledonous and four monocoryledonous species representing nife plant families.

The following species were treated: Beta vulgaris is ugar wet), Brassicg napus (oilseed rape), Cucumis sativus (cucumber), Fagopyrum esqulentum (buckwhear), Glycine mase (soybean), Lactuca sativa (lettuce), Licopersicon esculentom (topato), Allium cepa (conon), Avena sativa (oat), Lolium perenne (ryegrass) and Zea mans (com).

Five seeds of each species were sown in pots montained in the glasshouse. The soil surface of the pots were treated once with NVI 02900 SL 200 g/L using a laboratory track sprayer applied at 410 g a.i./ha and a volume rate of 200 L/hg. Each pot (replicate) contained 5 seeds and there were 40 seeds treated i.e. 8 replicates. Control pots were treated with de-ionized water.

Following application, nots were grown and maintained under glasshouse conditions with a temperature control set at 23 ± 8°C during day and 18 28°C at hight with a 16 h photoperiod.

The blank control was 200 L deignized water ha. Seedlings were exposed to the test item for 21 days.

### 3. Observation and measurements:

The parameters measured were entergence, survival of the emerged seedlings, visual phytotoxicity, plant growth stage, shoot length and shoot dry weight. Observations were recorded 7, 14 and 21 days after application and assessments were made against the water treated controls.

### 4. Statistical analysis

Statistical analysis of data was performed to obtain significance for shoot length and shoot dry weight effects, carried out using the Pairwise Mann-Whitney-U-Test (one sided smaller;  $p \le 0.05$ ) by ToxRat statistics.



### RESULTS AND DISCUSSION

### D. Environmental Parameters

Pots were kept in glasshouses under conditions as described above (see material and methods).

### E. Biological Findings

As a result of a soil application of BYI 02960 SL 200 g/L with 410 g a.i./ha/his study revealed a very low level of phytotoxicity. There were no adverse effects of surviving with any of the species tested. There were limited phytotoxic symptoms in this study with slight necrosis, leaf deformation and stunting in Buckwheat, Soybean, Tomato, Oat and Com (maize).

Buckwheat (Fagopyrum esculentum) was the most sensitive species for shoot length and shoot dry weight, with 13.8% and 19.7% reduction, respectively, which were both statistically significant.

A summary of the findings from a single application of 410 a.i./harto eleven plant species tested is summarised in the following table:

Plant Species	Emergence (% inhibition)	Sprvival (% inhibition)	Phytotoxicity (	Skoot Cength (Coeduction)	Shoot Dry ↓ Weight (% inhibition)
		"Dicotyled	ineae (V ZV		√
Beta vulgaris	1 <b>5.</b> %	<b>%</b> 5.1		Q 1.8 °	-7.4
Brassica napus	2.8		0 ~	, 1.3 ©	-10.5
Cucumis sativus	°≫-2.9 «	© 06		-5.3°	-8.6
Fagopyrum esculentum			- 40%	13,8	19.7
Glycine max	(8.3		ॐ 0 - <b>3</b> 90%	7.0	10.7
Lactuca sativa	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	% % %		2.9	1.1
Lycopersicon esculentum			0 - 20	-2.0	-16.3
- E	o v	🔬 Mondçotylei	doneae 🔍		
Allium cepay √	-3.3	©"-3.4 <sub>~</sub>		3.6	6.5
Avena satiya	, Ø 7 <b>5</b> )		  ï - 20,  ©	0.1	-1.0
Lolium perenne	) £J2.5 &		(K) (K)	4.6	7.4
Zea mays	7.5	~~~ 0~~	O 0≥20%	4.2	13.1

Bold figures for shoot dry wight are statistically significant Pairwise Mann-Whitney-U-test, one sided smaller;  $p \le 0.05$ )

### F. Validity Criteria

This study can be considered valid as the valuatity criteria of crop specific emergence and at least 90% survival of the emerged seedlings during the study period was achieved for the untreated controls of all species tested.

### CONCLUSEON

Following soil surface application of BYI 02960 SL 200 g/L applied at 410 g a.i./ha (corresponding to 2.4 kg product ha) to deven terrestrial non-target plant species, no adverse effects on emergence, seeding surfaval, visual phytotoxicity, growth, shoot length and shoot dry weight above 25% effect were observed in this seedling emergence and growth study. Only minimal responses were observed, typically within the range of natural variability.

### IIA 8.13 Effects on terr. vertebrates other than birds / wild mammal toxicity

Not a current data requirement, therefore no additional studies have been performed.

### IIA 8.14 Effects on other non-target organisms believed to be at risk

Chronic studies on *Folsomia* and *Hypoaspis* have been conducted with the formulated product. BYI 02960 SL 200G and are summarized below for the parent and for the metabolites DFA and CNA.

Report:	KIIA 8.14/01;,72009
Title:	BYI 02960 SL 200 G: Influence of the Reproduction of the Collembola species
	Folsomia candida tested in Arthucial Soil with % Peat°
Report No:	FRM-COLL-75/09
Document No:	M-359728-01-2 & & & & & & & & & & & & & & & & & & &
<b>Guidelines:</b>	ISO 11267 (1999)
<b>Deviations:</b>	To fulfil the recommendations of the proposal for a new QECD guideline 5%
	peat instead of 10% peat in the artificial soil was tested
GLP:	Yes (certified laboratory)

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the chronic effects of BYI 02960 SL 200 © (Sample description: FAR01438-00 (Batch ID: 2009-001253; Material No.: 79718845, Specification No.: 102000021884; purity 199.8 g BYI 02960/L 17.0% w/wyto springtails (Folsomia candida).

Ten springtails (10 to 12 days ofd) perceplicate (5 replicates per treatment group) were exposed in an artificial soil system with a peat content of \$% over a period of 14 days to nominal concentrations of 8.8, 13.2, 19.9, 29 and 40 mg test item/kg artificial soil droweight corresponding to 1.5, 2.3, 3.4, 5.1 and 7.6 mg and /kg droweight soil in the 15 run and 5.88, 7.06 and 8.47 mg test item/kg dry weight soil, corresponding to 5.00, 120 and 1.44 mg a.i./kg dry weight soil in the 2<sup>nd</sup> run. Since the first test run on BYI 02960 SL 200 G did not provide a final result, a second test on was performed studying lower concentrations. In addition a water control was tested.

Mortality and reproduction were determined after 28 days

The overall 28-day NOFC was determined to be 8.47 mg product/kg soil dry weight.

### MATERIAL AND METHODS A. Materials

### 1. Test material

Test item: PyI 02960 SL 200 G

Chemical state and description. Clear dark brown, liquid

Specification No.: 102000021884

Sample description: FAR01438-00

Batch row: 2009-001253

Nominal content of active incredient: BYI 02960: 200 g/L

Analytical content of active BYI 02960: 17.0% w/w, 199.8 g/L according to certificate of analysis

ingredient.

Density: 1.175 g/mL at 20°C

Stability: Expiry date: 2010-03-20, when stored at room temperature

### 2. Vehicle and/or positive control

Test item mixed with: Water

Controls: Water control as negative control

### 3. Test organisms

Species: Folsomia candida

Common name: Springtail

Source: In-house lab culture Age at study initiation: 10 to 12 days old

Maintenance of culture:

Temperature:  $22 \pm 2^{\circ}$ C Photoperiod: Permanent dark

Food: Once a week onth bakers dry yeast

### B. Study design and methods

1. In life dates June 30 to September 18,200

### 2. Design of biological test

Springtails (Folsomia candida; 10 to 2 days old) were exposed to BYI 0 960 St 200 C; (content of a.i.: 199.8 g BYI 02960/L; 17.0% www) in an artificial soil system with 3 % peat over a period of 4 weeks.

Since the first test run on BY192960 SL 200 G did not provide a final result, assecond test run was performed studying lower, concentrations

10 Collembola (10-12 days old) per replicate  $\circ$  replicates per treatment group) were exposed to control (water treated), 8.8, 13.2, 19.9, 29.8 and 44% mg test item/kg artificial  $\circ$ il dry weight corresponding to 1.5, 2.3, 3.4, 5.1 and 7.6 mg/a.s./kg dry weight soil in the first est run and 5.88, 7.06 and 8.47 mg test item/kg dry weight soil corresponding to 1.00 1.20 and 1.44 mg ac./kg dry weight soil in the second test run. In addition, a water control was tested as negative control. Each jar (glass vessels; 140 mL; 5 cm in diameter) served as one replicate filled with 30 g wet weight test substrate. 10 springtails were used per replicate. The test was conducted with 5 replicates per treatment level. The test was conducted at  $20 \pm 2$ °C and 577 to 608 lux at 16 h light. 8 h dark. The artificial soil contained 5% peat, 20 % kaolinite clay, 74.8% quartz sand and 0.2% calcum carbonate.

### 3. Observation and measurements

After 28 days mortality of the adult springfails and the number of juveniles were assessed as indicated below in the result section.

### 4. Statistical analysis

The software used to perform the statistical analysis was ToxRat Pro 2.09, (2006). Data of reproduction were tested for normal distribution and homogeneity of variance using Kolmogorov - Smirnov -Test and Cochran -Test ( $\alpha$  = 005) 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.



### RESULTS AND DISCUSSION

### A. Physical and Chemical Parameters

Table: pH and water content of the artificial soil

Test item	p	Н	Water cor	ntent in %	≫ WH	Cmax
concentration <sup>1)</sup>	Start	End	Start	End	Start	And S
first test run					WHO WHO	Cmax <sup>2)</sup>
control	5.64	5.36	20.59	18.82	<i>,</i> 48.21	6 43, <b>0</b> 9
8.8	5.74	5.37	20.47	a 18.49	47.85	7 42×16
13.2	5.69	5.34	20.78	7 18.45 Q	් 48.74 ලී	~2.06, ©
19.9	5.69	5.35	20.57	18.60	48.13	\$ 42.46
29.8	5.67	5.33	20.53	18.03	48.00	40, Ø
44.6	5.69	5.32	19.96	19.38	o° 46.85 4	44.69
second test run			Q)	~ .0	WHO	Cmax Q
control	5.82	5.71	€ 19.05 ©°	\$\frac{9}{7.42}\tilde{}	47.780°	42.82
5.88	6.05	5.68	©"19.3 <i>3</i> ©"	≥ 17.6 <b>0</b>	© 48 <b>/9</b> 5	43.37
7.06	5.90	5.66	19,43	17029	¥8.96 <b>○</b>	<b>45</b> 2.45
8.47	5.95	5.66 🐇	19k20 N	≥1,7.01 ≥4,	Ø¥8.25,	<sup>∞</sup> 41.62

<sup>1)</sup> mg test item/kg soil dry weight

### **B.** Biological Findings

Observations on mortality and number of juveniles are listed as follows:

Table: Effects of BYI 02960 SI 200 G on mortality and reproduction of Followina candida

	<i>∞</i> % 1	~~ .	- N
Concentration (mg prod/kg. dws)	adult mortality (%) 🖒	mean number of	reproduction 🖖
(mg prod/kg. dw🔊		juvepites ± SD	(as % of control)
control (2nd ran)		12002 ± 100V	-
5.88		(√	107.3
7.06	14	13000 71	108.9
<b>8.4</b> 7		1166 ± 124	97.0
control (1st run)		\$40 ±60	-
8.8		<u> </u>	82.9*
13.2	\$\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2	934 ± 144 *	69.7*
19.9	122	680°± 130 *	50.8*
29.8		27 ± 169 *	31.9*
44.60	×44 × ×	196 ± 61 *	14.7*

<sup>\*</sup> Significantly different from the control (Walkam's Test, one-sided-smaller,  $\alpha = 0.05$ )

### C. Validity Criteria,

The validity criteria (< 20% control mortality: average reproduction rate > 100 juveniles per control vessel and variation of reproduction 50% Q.) are fulfilled.

# D. Test with toxic reference substance

Reference Gubstange: Betosip, active ingredient: Phenmedipham (153 g/L)

ate of phost recent test 2009

Result: O NOEC 7.6 mg a.i./kg artificial soil dry weight

<sup>&</sup>lt;sup>2)</sup> % WHC<sub>max</sub> = percent of maximum water Golding vapacity of 53.8 g water per 100 g artificial soft dry weight

<sup>3) %</sup> WHC<sub>max</sub> = percent of maximum water holding capacity of 49.25 gwater per 100 gartificial soil droweigh

### E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

NOEC: 8.47 mg test item/kg dry weight soil (equivalent to 1.442 mg a.i./kg dws) LOEC: 8.8 mg test item/kg dry weight soil (equivalent to 1.498 mg@i./kg dws)

### **CONCLUSION**

The chronic effect of BYI 02960 SL 200 G on springtails (Folsomia candida) can be quantified as an overall-NOEC of 8.47 mg product/kg dry weight soil, equivalent to 1.442 mg a.i./kg dry soil

Report:	KIIA 8.14/02, (2010) (2010)
Title:	Metabolite BYI 02960-diffuoroactic acid. Influence on the reproduction of the collembolan species <i>Folsomia candidg</i> tested in artifical soil
Report No:	FRM-COLL-85/10
Document No:	M-368675-01-2 0
Guidelines:	OECD Guideline No. 282
<b>Deviations:</b>	None & & & & & & & & & & & & & & & & & & &
GLP:	Yes (certified laboratory) & S O O S

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the chronic effects of diffuoroacetic and (Qingin Batch No: BCOO 5984-1-1; Batch code: BCS-A \$56716-01-0 TOX \$889-00; purity 95 \$ w/w) on the reproduction of springails (Folsom Candida).

Springtails (10 to 12 days old) were exposed in an artificial soft system with peat content of 5% over a period of 14 days to a concentration of 100 mg/kg. Ap addition a quartz sand control was tested.

Mortality and number of juveniles were used to determine the endpoints.

The overall 28-day I

BYI 0200-diffuoroacetic acid (DFA, code: BCS-AA56716) Test item:

Substance, technical (pure metabolite) Type of test material:

Colourless higuid

Chemical state and description
Origin Batch number: BCOO 5984-1-1 (Batch code: BCS-AA56716-01-01)

XOX 08889-00 Sample description: CAS#: Purity: 95.8% w/w

Stability Expiry date: 29.07.2010, when stored at  $+25 \pm 5$ °C

Test item to xed with: Quartz sand Controls: Water control

### 3. Test organisms

Species: Folsomia candida

Common name: Springtail

In-house lab culture since April 2002; originally obtained from Source:

**IBACON** 

Age at study initiation: 10 to 12 days old

Maintenance of culture:

 $22 \pm 2$ °C Temperature: Photoperiod: Permanent dark

Food: Once a week with bakers dry yeast

### B. Study design and methods

1. In life dates March 26 to April 2

### 2. Design of biological test

nacetic acid (code:

The concer

sand cor

The concer

The concer Springtails (Folsomia candida; 10 to 12 day old) were exposed to difluoroacetic acid Code &CS-AA56716; purity 95.8 %) in an artificial soil system over a perfod of 28 days. The concentration was 100 mg/kg (limit test) mixed into the sol. In addition a water treated quartz sand control was tested. Each replicate consisted of a jar (glass; 140 mL) filled with 30 g wet weight soil. Ten collembola were used per replicate. The test was conducted with replicates per treatment level. The test was conducted at 20 ± 2°C and 692 to 702 lux of 16 h light: 8 h dark? The strtificial soil containe? 5% peat, 20% kaolinite clay, 74.85% quartz sand and 0.15% calcham carbonate.

### 3. Observation and measurements

Food (2 mg granulated try years) was amended weekly during food weeks. After 4 weeks mortality of and reproduction were assessed as ordicated below in the result section.

The software used to perform the statistical analysis was FoxRat Pro 2009 released November 08, 2006, 2006). Data of reproduction were tested for normal distribution and homogeneity of variance using Kolfragorov - Smarnov Sest and Cochran's Jest (6 0.050) respectively. Data of reproduction were normally distributed and homogeneit of variances was given. Therefore Student-t test (one-sidedsmaller,  $\alpha = 0.05$ ) was used to determine

### RESULTS AND DISCUSSION

# A. Physical and Chemical Pagmeters

The soil pH was 5.5 To 5.65 Th was 42.39 to 49.33% of the maximum water holding capacity.

### B. Biological Findings

Observations on mortality and reproduction are listed as follows:

In the control group 2.5% of the adult Folsomia candida died which is below the allowed maximum of  $\leq 20\%$  mortalis. An  $50_{50}$  could not be calculated and is considered to be > 100 mg test item/kg artificial soil dry weight.

Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller,  $\alpha = 0.05$ ) revealed no significant difference between control and the treatment group.

Table: Effects of difluoroacetic acid on mortality, body weight change and reproduction of *Folsomia candida* 

Concentration of test item (mg p.m./kg dry soil)	Adult mortality (%)	Mean number of juveniles ± SD	Reproduction (as % of control)
control	2.5	$1337 \pm 175$	
100	1.3	$1268 \pm 146$	95 % (n.s.)

### C. Validity Criteria

Validity criteria	Recommended by the Obtained by thi	s <b>zo</b> jdy
Mean adult mortality	$\leq 20\%$ $\approx$ $\approx$ $2.5\%$	y C
Mean number of juveniles per replicate (with 10	≥ 100 % 1337	Į
colembolans introduced)		°~
Coefficient of variation calculated for the	$0^{2} \le 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30^{2} = 30$	۰ .۱
number of juveniles per replicate		Y 5

All validity criteria were met. Therefore this study is wild

# D. Test with toxic reference substance

Reference substance:

Date of most recent test:

Boric acid

March 2010

Result: EC 96 mg boric acid/kg soil dry veight

NOEC<sub>repress</sub>44 m@boric acid/kg soil dry weight

# E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

LC 50 mortality
NOEC reproduction:
LOEC reproduction
Overall NOEC

100 mg test item/kg soil dry weight

100 mg test item/kg soil dry weight

100 mg test item/kg soil dry weight

100 mg p.m./kg.oil dry weight

### CONCLUSION

The chronic effect of difluoroace acid (BCS-AA56746) on springtails (Folsomia candida) can be quantified as an overall NOEC of > 100 mg/s/m./kg soil dry weight.

Report:	(2010)
Title:	To-chloronicotific acid (AE F161089): Influence on the reproduction of the
	collembolan species <i>Folsomia candida</i> tested in artificial soil
Report No:	PRM-Coll-111/11
Document No.	- 107-407861-01-2
Gordelines	OECD Guideline No. 232
Deviations:	None
GLP: <sup>U</sup>	Yes (certified laboratory)

### **EXECUTIVE SUMMARY**

The aim of the study was to determine the chronic effects of 6-chloronicotinic acid (Origin Batch No: M12653; Batch code: AE F161089 00 1B99 0001; purity 98.8% w/w) on reproduction of springtails (*Folsomia candida*). Ten springtails (10 to 12 days old) per replicate were exposed in an artificial soil system with peat content of 5% over a period of 28 days to concentrations of 106 mg/kg (1st rum) and 10, 17, 30, 52 and 90 mg test item/kg artificial soil dry weight (2nd run). Eight replicates per dose for the first run and 4 replicates for the second run were performed. In addition a water control was tested. Mortality in the adults and number of juveniles were determined after 28 days. The overall 28 day NOEC was 90 mg/kg soil dry weight.

### MATERIAL AND METHODS

### A. Materials

### 1. Test material

Test item: 6-cl-foronicorinic acid (BYI 92960 6-CNA AE FI 61089.

Type of test material: Sympstance technical (pure metabolite)

Chemical state and description: Peige powder

Origin Batch number: Q M12653 (Batch code; AE FL 1089 00 1B9 0001)

CAS#: 5325-23-80 Purity: 98.8%, w.w.

Stability of test compound: Expire date: 99/07.2012, when stored at + 5 \(\frac{1}{2}\)5 °C

### 2. Test solutions

test item mixed with: Quart sand Quart sand

### 3. Test organisms

Species:
Common name:
Source:
Age at study initiation:
Maintenance of culture:

Maintenance of culture:

Age at study initiation:

Maintenance of culture:

Maintenance of

Temperature: 22 ± 22 ± 22 ± 22 ± 22 ± 24 Photoperiod: Peggo

Food: Once a week with bakers dry yeast

# B. Study design and methods

1. In life dates January 14 to April 15, 2011

### 2. Design of bological test

Springtails Folsonia candida; 10 to 12 days old) were exposed to 6-chloronicotinic acid (code: AE F161089; purity 98.8% www) in an artificial soil system over a period of 28 days. Concentrations were 100 mg/trg (1st min) and 10, 17, 30, 52 and 90 mg test item/kg artificial soil dry weight (2nd run). In addition, a water control was tested. Each replicate consisted of a jar (glass; 140 mL) filled with 30 g wet weight. 10 springtails were used per replicate. The test was conducted with 8 (1st run) and 4 (2nd run) replicates per treatment level. In the controls, 8 replicates were tested. The test was conducted at

 $20 \pm 2$ °C and 574 to 614 lux (1<sup>st</sup> run) and 567 to 590 lux (2<sup>nd</sup> run) at 16 h light: 8 h dark. The artificial soil contained 5% peat, 20% kaolinite clay, 74.8% quartz sand and 0.2% calcium carbonate.

Directly after the addition of the collembolans, they were fed with granulated dry yeast. Feeding was also done 14 days after test start.

### 3. Observation and measurements

The surviving adults and living juveniles were counted. Missing adults (compared to the number of initially placed test organisms) were considered to be dead, since dead collembolans cannot be extracted. Endpoints of the test were mortality of the adult collembolars in comparison to the initially placed test organisms expressed in % and the number of offspring hat the difference of offspri until the end of the test period per test vessel (reproduction).

### 4. Statistical analysis

The analyses were done with the program Tox Rat Professional 2.10 reproduction were tested for normal distribution and nomogeneity of variance using Komogowov Smirnov -Test and Cochran's -Test ( $\alpha = 0.05$ ) respectively. In the 1st test run Pata of reproduction were normally distributed and homogeneity of variances was given. Therefore Student of test one-sidedsmaller,  $\alpha = 0.05$  was used to determine NOEC and LOEC values. In the  $2^{\text{nd}}$  test run data of reproduction were normally distributed and homogeneits of variances was given. Therefore William's-t test, one-sided-smaller,  $\alpha = 0.05$  was used to determine NOEC and POE

### RESULTS AND DISCUSSION

### A. Physical and Chemical Parameters

The soil-pH was 5.47 to 5.86. The water content capacity 6 of the maximum water holding capacity.

### B. Biological Findings

Observations of mortality and number of juvenile are listed as follows

Effects of AE R161089 on mortality, body weight change and reproduction of Folsomia candida Table:

Concentration	. V	Adult mortality (%)	Mean number of	Reproduction
(mg/kg dry soil)	~Ó)		V • (/o	(as % of control)
			© deviation	
control (1st run)	<del>-</del> Q	13/88	$15\sqrt{1.4} \pm 165.6$	
100	, Ö	20.0	$1258.9 \pm 132.5$	81.7*
control (2nd run)		2.5	$\bigcirc 1566.9 \pm 166.0$	
90		5.07	1485.8 ± 124.8	94.8 n.s.
52	°~√	Q 5.0 &	$1434.3 \pm 155.0$	91.5 n.s.
30 🚀	, Ş	A 7.5 %	$1573.5 \pm 95.4$	100.4 n.s.
17 😽		\$\frac{1}{2}\forall 5.0  \text{\$\infty}	$1641.3 \pm 51.4$	104.7 n.s.
10	₹. ◊	50	$1583.0 \pm 93.6$	101.0 n.s.

<sup>\*</sup> Significantly different to control (Student's t-test, one-sided smaller,  $\alpha \le 0.05$ )

# C. Validit Critonia

All validity conteria were thet. Therefore this study is valid.

Validity criteria	Recommended by the guideline	Obtained in this study	
		1 <sup>st</sup> run	2 <sup>nd</sup> run <sub>@a</sub> °
Mean adult mortality	≤ 20 %	13.8 %	2.5 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1541	1567
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	10.7 %	10.6 %

### D. Test with toxic reference substance

Reference substance: boric acid
Date of most recent test: March 2011

Result: EC<sub>50</sub>: 91 mg borge acid/kg soil dry weight

NOEC<sub>repro</sub>: 44 mg boric acid/kg soil dry weigh

### E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

LC<sub>50</sub> (adult mortality)
LOEC reproduction

100 mg p.m./kg soil dry weight

### **CONCLUSION**

The chronic effect of 6-chloronic tinic acid (AEF16,089) of springtails (Folsonia candida) can be quantified as an overall NOE of 90 mg p.m. /kg soil dry weight based on the hight but statistically significant effects son at 100 mg pm/kg.

Report:	KMA 8.14/04; A. 2009
	KMA 8.14/04; A. 2009
Title:	BYI 02960 SL 200 G: InDuence on morcolity and reproduction on the soil mite
	Species Aypoaspis aculeifer tested in artificial soil with 5% peat
Report No:	Kra-19409 & A
Document No:	M-358752-01-2 V O O
Guidelines:	OECD Guideline No. 226 (2008)
<b>Deviations:</b>	To fulfil the recommendations of the proposal for a new OECD guideline 5%
	peatinstead of 10% peat in the artificial soil was tested.
GLP:	Yes (certified laboratory)

### EXECUTIVE SUMMARAT

The aim of the study was to determine the chronic effects of BYI 2960 SL 200 G, (Sample description: FAR01438-00 (Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 102000021884); 17.0% w/w/fo predatory soil mites (*Hyppaspis aculeifer*).

Ten mites (28 days old, after start of egg-laying) per replicate (4 replicates per treatment group and 8 control (replicates) were exposed in an artificial soil system with a peat content of 5% over a period of 14 days to reminal concentrations of 100, 178, 316, 562 and 1000 mg test item/kg artificial soil dry weight. In addition, a water control was tested. Mortality of the adults and number of juveniles were used to determine the endpoints.

The overall 14-day NOEC was determined to be  $\geq$  1000 mg product/kg dry weight soil.

### MATERIAL AND METHODS

### A. Materials

### 1. Test material

Test item: BYI 2960 SL 200 G

Type of test material: Formulated product (soluble (liquid) concentrate)

Chemical state and description: Dark brown, clear liquid Specification No.: 102000021884

Material number: 79718845
Sample description: FAR01438-00
Batch No.: 2009-001253
Nominal content of active substance: BYI 02960: 2009

Analytical content of active BYI 02960: 17.0 % w/w, 1998 g/L according to certific

substance:

Solubility: In water: Soluble Density: 1.175 g mL at 20°C

Stability: Expity date: March 10, 2010 wheat stored at +25 \(\frac{2}{3}\) of

### 2. Vehicle and/or positive control

Test item mixed with: 

Water

Controls: Water combol as pogative Control

### 3. Test organisms

Species: Hypoaspis aculeiff
Common name: Soil mite Soil mite

Source: In-howe lab Atture

Age at study initiation. 28 days after start of egg-laying

Feeding during test Fed with sheese mites or days Q4, 7 and 10

Maintenance of Calture,  $0 \le 20 \pm 2^{\circ}$ 

Food: Sheese mites broad on knower's weast

### B. Study design and methods

1. In life dates @ . O August 13 @ September 14. 200

### 2. Design of biological test

Predatory soil mites (Hypoaspi aculeifer; 28 days after start of egg-laying) were exposed to BYI 2960 SL 200 G; (content of a.i.: 140% w/w) in an artificial soil system over a period of 14 days. Cheese mites were amended twice a week.

10 fertilized female mites per explicate (4 replicates per treatment group) were exposed to concentrations of 100, 178, \$16, 562 and \$6000 mg test item/kg dry weight artificial soil. In addition a water control was ested in 8 replicates.

Each jar (glass) essels 140 mL) served as one replicate filled with an amount equivalent to 20 g dry weight artifical soil 5% peat, 20% kaolinite clay, 74.8% quartz sand and 0.2% calcium carbonate). The depth of the soil layer was approximately 1.5 cm. The test was conducted at  $20 \pm 2$ °C and 575 to 585 lux at 16 h light; 8 h dark.

### 3. Observation and measurements

After 14 days mortality of adult mites and number of juveniles were assessed as indicated below in the result section.

### 4. Statistical analysis

The software used to perform the statistical analysis was ToxRat Pro 2.10 (released February 49) , 2001-2009). Data of reproduction were tested for normal distribution and homogeneity of reproduction were normally distributed and homogeneity of variances was given. The fore williams test (one-sided smaller,  $\alpha = 0.05$ ) was used to determine NOEC and LOEC values

### RESULTS AND DISCUSSION

### A. Physical and Chemical Parameters

reproduction were normally distributed and homogeneity of variances was given. Therefore williams test (one-sided smaller, $\alpha = 0.05$ ) was used to determine NOEC and LOEC values.									
test (one sided smaller $\alpha = 0.05$ ) was used to determine NOEC and LOEC values $\frac{1}{2}$									
test (one-sided smarter, $\alpha = 0.05$ ) was used to determine NOEC and FOEC values.									
A. Physical and			<b>%</b>						
Table: p	H and water	content of t	he artificial						
Test item concentration	pН	(	Water conto	ent in %		% WHE	max2,\$)		
1)	Start	End O	Start	End S	0% 5 deylation (	Start S.	© Ænd		
Control	5.84	5.68 J	© 19 <i>2</i> \$	(19.400)	<b>₹</b> 0.79 <b>₽</b>	A8.40	48.88		
100	5.74	× 80 ×	19.05	J 19 <b>.62</b>	3.01	<b>3</b> 47.78	49.56		
178	5.72	≪"5.63	ر 9.26 الأراث	19.75	2,54	48.43	49.96		
316	5.63	5.53	\$\infty 19.40\mathcal{O}''	18.89	Q.59 ~	48,86	47.29		
562	5.56	\$.48	19.43	√ 19.3 <b>⊘</b>	-0.33	≈ <b>3</b> 8.97	48.77		
1000	5.62	\$3.51 \Q	19.04 🎘	19.92	148	<u></u> 947.75	48.62		

<sup>1)</sup> mg test item/kg soil dr weight

### B. Biological Finding

Observations on mortality and comber of juveniles are listed as follows:

Table: Effects of BY1 2960 St 200 Con mortality and reproduction of Hypoaspis aculeifer

Concentration	a Guilt mortality (%)	mean number of	reproduction
(mg test item/kg dws) 🔘	* \$ ( <b>%</b> ) * }	Quveniles	(as % of control)
control control		$361.9 \pm 23.4$	-
100	2.5 0	$334.5 \pm 44.8$	92.4
<del>1</del> 78	5 5 6	$377.3 \pm 22.8$	104.2
<b>3</b> 16	Q 0.0 &	$387.5 \pm 23.1$	107.1
562	A 20.0 7 3	$384.3 \pm 19.3$	106.2
1000	$\mathcal{S}' \mathcal{S} 0.0 \mathcal{Q} \mathcal{S}'$	$382.0 \pm 43.4$	105.6

### C. Validity Criteria

The validity of terial 20% control mortality; average reproduction rate > 100 juveniles per control vessel and variation of reproduction < 30% c.v.) are fulfilled.

<sup>1)</sup> mg test item/kg soil draweight weight woulding capacity of 4925 g H<sub>2</sub>O/100 dry weight artificial soil

<sup>3)</sup> The results represent rounded values calculated on the exact raw data

### D. Test with toxic reference substance

D. Test with toxic refe	rence substance
Reference substance:	dimethoate
Date of most recent test:	09 FEB 2009
Result:	LC <sub>50</sub> of 3.86 mg a.i./kg dry weight artificial soil
E. Biological Endpoin	ts Derived
From the results presen	dimethoate 09 FEB 2009 LC <sub>50</sub> of 3.86 mg a.i./kg dry weight artificial soil  ts Derived  ted above the following biological endpoints can be derived:  >100 mg product/kg dws >1000 mg product/kg dws ≥1000 mg product/kg dws ≥1000 mg product/kg dws  NOEC of ≥ 1000 mg product/kg dw weight soil.
LC <sub>50</sub> :	>100 mg product/kg dw
LOEC (reproduction):	>1000 mg product/kg dws
NOEC (reproduction):	≥1000 mg product & dws
` <b>-</b>	
CONCLUSION	YI 2960 SL 200 G on predatory soil in tes (As poasors acutaifer) can be
CONCLUSION	
The chronic effect of B	YI 2960 SL 200 G on predator soil in tes (Appoasors acute if er) can be
quantified as an overall	-NOEC of ≥ 1000 mg product/kg dey weight soil.
	-NOEC of ≥ 1000 mg/product/kg dev weight soil.
Report:	KIIA 8.14/05, A. (2019)
Title:	BYI 2960-DFA BCS-A-5671 O. Influence on prortality and reproduction on the
	soil mure species Hypoaspis aculeifer to sted in artificial soil with 5% peat
Report No.:	kra-HR-27/40 D V V V
Document No.:	₩39009 F-01-2 Ø Ø Ø Ø Ø Ø Ø Ø Ø Ø Ø Ø Ø Ø Ø Ø Ø Ø Ø
Guidelines:	OECP 226 S
Deviations:	None S S S S
GLP:	Ves (certified laboratory)
EXECUTIVESUMM	
raruutivia Mulviyo	

The aim of the study was to determine the effects of diffuoroacetic acid (Origin Batch No: BCOO 5984-1-1; Batch code: BCS-4, 567 601-0 TOX,08889 00; Purity 958% w/w) on mortality and reproduction of predatory soil mites (Hypodspis gculeifer).

Ten female mites per replicate (3 and 28 days after start of egg-laying in the 1st and 2nd run, respectively) were exposed in an artificial soft system with peat content of 5 % over a period of 14 days to concentrations of 63 25, 250, 500 and 1000 me test item/kg dry weight artificial soil (1st run), respectively and 1000 mg test item/kg dry weight artificial soil (2nd run).

of javening of jav Mortality of adults and number of juveniles were determined.

The overall 14 day- NOEC was \$1000 mg test tem/kg dry weight artificial soil.

John difluoroacetic acid (BCS-AA ace technical (pure metabolite) aurless liquid COC 5984-1-1 (Batch code: BCS-AA5671; TOX 08889-00) 381-73-7 95.8% w.w. Expiry date: 29.07.2010, when stored at +25 ± 5 °C. Secretary of the state of the s THON I SO THE STATE OF THE STAT

### 2. Test solutions

Test item mixed with: Ouartz sand Controls:

### 3. Test organisms

Species:

Common name:

Source:

Age at study initiation:

Feeding during test:

Maintenance of culture:

Temperature: Photoperiod:

Food:

### B. Study design and methods

1. In life dates

### 2. Design of biological test

soil mite
In-house lab culture
35 and 28 days after start of egg-laying in the 1st and 2nd reorespectively
Fed with cheese nates on days 0 \$\frac{1}{2}\$, 7 and 11 (1st fun) and on days 0, 3, 7 and 11 (2nd fran)

10 \pm 2°C

ermanent dark

heese unites bred on brewer's yeast

to May 5, 2010

peospis aculeifer: 35 and 28 days after start of sed to difluoroacetic acid code:

pil (1st fax) Fertilized female predatory soft mites (Hypodspis acculeifer: 35 and 28 days after start of egg-laying in the 1st and 2nd run, respectively) were exposed to difluoroacetic acid code: BCS-AA56716; purity 95.8 % w/w) in an artificial soil system over a period of 14 days. Concentrations were 3, 125, 250, 500 and 1000 mg test item/kg dry weight artificial soil (1st run) and 1000 mg test item/kg dry weight artificial soil (2<sup>nd</sup> run). Since of the 1st test run in each concentration at least incone replicate more than 10 adult mites were found the test was repeated as limit test with 1000 mg test item kg dry weight artificial soil. In addition water control was lested. Fach replicate consisted of a jar (glass vessels; 140 mL) filled with equivalent to 20 g dry weight artificial soil. The depth of the soil layer was approximately 1.5 cm. Ten fertilized females in tes were used per replicate. The test was conducted with 4 replicates per treatment level. In the controls & replicates were tested. The test was conducted at  $20 \pm 2^{\circ}$ C and 400to 800 Lux at 16 h light: 8 h darle The artificial woil contained 5 % peat, 20 % kaolinite clay, 74.8 % quartz sand and 0.2 % cocium carbonate. After a period of \$4 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution 20 % ethyler glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All Hypoaspis aculeifer were counted under a binocular.

### 3. Observation and measurements @

Cheese mites were amended twice a week. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution 60 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All Hypoaspis aculeifer were counted under a binocular.

### 4. Statistical and ysis

The software used to perform the statistical analysis was ToxRat Pro 2.10 (released February 19, 2009); 2001-2009). Data of reproduction were tested for normal distribution and homogeneity of variance using Kolmogorroff-Smirnov Test and Cochran-Test ( $\alpha = 0.05$ ), respectively. Data of

reproduction were normally distributed and homogeneity of variances was given. Therefore Williams test (one-sided smaller,  $\alpha = 0.05$ ) was used to determine NOEC and LOEC values.

test (one-sided sn	naller, $\alpha = 0$	0.05) was us	sed to determine NOEC and LOEC values.
RESULTS AND	DISCUSSI	ION Parameters	s capacity (WHC <sub>max</sub> ) of the artificial soil used in this study were the artificial soil
71. I nysicai and	chemicai i	arameters	
Measured pH val	ues and wat	er holding c	capacity (WHC <sub>max</sub> ) of the artificial soil used in this study were
as follows:			
Table n	H and water	content of t	capacity (WHC <sub>max</sub> ) of the artificial soil used in this study were the artificial soil
таыс. р	II and water	content of t	
mg test item/kg	p	Н	% water content % of WITC mas
dry weight artificial soil	Start	End	Start End deviation Start End
First run			
Control	6.25	5.80	17.60 17.160 42.07
63	6.09	6.14	1298 1769 2 - 1.62 244.512 49.63
125	5.81	5.71,°°	20.55 2 L911 V - 20 S 52.52 Q 47.96
250	5.61	5.50	19.31 18.68 3 46.65
500	5.56	<i>5</i> ,4 <sup>7</sup> ≼	20 4 18.25
1000	6.00	≈©5.43 °>⁄	19.82 18.74 7 - 5.4 50.20 46.84
Second run		** &	
Control	5.51	5. <b>Q</b> °	19.22 20.30 25.6 3 48.30 51.72
1000	5.50	<b>4</b> .87	19.63 7 19.52 - 2.60 7 9.61 48.00

### B. Biological Finding

Biological observations are listed in the following table

Effects of BVI 92960-DFA (BES-AA56716) on mortality, body weight change and Table: reproduction of Hyppaspis aculeifer

Concentration (mg p.m./kg dry soil)	Adult moreality (%) Mean number of juveniles ± SD	Reproduction (as % of control)
Control (1st run)	\( \sqrt{9} \) \( \sqrt{5} \) \( \sqrt{9} \) \( \	-
Control (2nd run)	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	-
63	$5.0 \pm 5.1$	106.3
125	$2.5$ $356.5 \pm 39.4$	96.4
250	-102 436.0 ± 43.6	117.9
500	$\sim$ -7 $\circ$ $\sim$ 377.3 ± 18.3	102.0
1000 (1st run)	393.3 ± 48.1	106.3
1000, (2ndt run)	391.9 ± 29.9	103.0

### C. Validity Criteria

Validity criteria (control values)	Recommended by the	Obtained in the 1 <sup>st</sup>	Obtained in the 2 <sup>nd</sup>
	y guideline	test run	test run
Mean adolt female mortality	≤ 20 %	5.0 %	1.3 %
mean number Djuventes per			
replicate (with 10 adolt females	≥ 50	369.9	380.6
introduced			
coefficient of variation calculated for			
the number of juvenile mites per	≤ 30 %	12.0 %	9.9 %
replicate			

All validity criteria were met. Therefore both test runs of this study are valid.

### D. Test with toxic reference substance

Reference substance: Dimethoate 03 FEB 2010 Date of most recent test:

Result: LC<sub>50</sub> of 4.2 mg p.m./kg dry weight soil

NOEC<sub>repro</sub>: 3.2 mg p.m./kg dry weight soil

From the results presented above the following biological endpoints can be derived NOEC:

LOEC:

### **CONCLUSION**

The chronic effect of difluoroacetic acid BCS can be quantified as an overall-NOECOf ≥ 1000 mg test item/kg dev

Report:	KIIAS8.14/06; A. (2011) & S
Title:	6-chiloronicotinic acid (AE \$161089): Influence on mortaliticand reproduction on the
	soft mite species Hypoaspis aculeifer tested in arithicial soft 🔣 🛴
Report No.:	kfa-HR <u>\$</u> 44/11
Document No.:	VM-40434-012
Guidelines:	OECD 226, 2008 Ø S S
Deviations:	None of the second of the seco
GLP:	Yes (contified laboratory)
	The regular of the sensitivity of the test organisms was not performed according
O' Š	to GLP.

The aim of the study was to determine the chronic effects of 6-chloronicotinic acid (Batch code: AE F161089 00 1B99 0001; purity \$8.8% w/w) on mortality and reproduction of predatory soil mites (Hypoaspis acyleifer

Adult mites (28 days after start of egg-laving) were exposed in an artificial soil system with peat content of \$\sigma\% over a period of \$\partial 4\$ days \$\sigma \text{o}\$ a concentration of 100 mg test item/kg dry weight artificial soil. In addition, a water control was tested. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing. All Hypoaspis aculeifer were counted under a binocular.

Mortality in the adults and number of juveniles were used to determine the endpoints. The overall 8was determined to be  $\geq 100^{\circ}$  mg p.m./kg soil dry weight.

### MATERIAL AND METHODS

### A. Materials

1. Test material

Test item: 6-chloronicotinic acid (BYI 02960-6-CNA, AE&I61089)

Type of test material: Substance, technical (pure metabolite)

Chemical state and description: Beige powder

Origin Batch number: M12653 (Batch code: AE F161089 00 1B29 0001)

CAS#: 5326-23-8 Purity: 98.8% w/w

Stability of test compound: Expiry date: 09.07.2012, when store  $2 \pm 5 = 6$ 

2. Test solutions

Test item mixed with: Quartz sand Controls: Water control

3. Test organisms

Species: Hypoaspis aculeito
Common name: Soil mile

Source: In-house lab culture

Age at study initiation: 28 days after start Pegg-loging

Feeding during test: feed with cheese notes on days 0, 2, 7 and 1

Maintenance of culture:

Temperature:
Photoperiod:
Permanent da

Food: Cheese mites been on brewer's weas

# B. Study design and methods

1. In life dates

January 14 to February 4, 2011

# 2. Design of biological test

Predatory soil mites (Hypoaspix culeifer; 28 days after start of egg Jaying) were exposed to 6-chloronic tinic acid (e.de: AF 161089; purity 98.8 %w/w) in an artificial soil system over a period of 14 days. One concentration of 100 mg test item/kg dry weight artificial soil was tested. In addition a water control was tested, Each replicate consisted of a jar (glass vessels; 140 mL) filled with the equivalent of 20 g dry weight artificial soil, the depth of the soil layer was approximately 1.5 cm. 10 fertilized females mites were used per replicate. The test was conducted with 8 replicates per treatment level. The test was conducted at  $20 \pm 20$  c and 640 to 739 lux at 16 h light: 8 h dark. The artificial soil contained 5% peat, 20% kaokinite clay, 74.8% quartz sand and 0.17% calcium carbonate.

Directly after the addition of Hypoaspis aculeifer, they were fed with the cheese mite Tyrophagus putrescentiae. Cheese mites were bred on brower's yeast in the laboratory. Feeding was also done 3, 7 and 10 days after test start. Between 0.050 and 0.090 mg food per test vessel was added per feeding date. Each test vessel was weighed for the determination of water loss. After 7 days the loss of water was determined by reweighing the test vessels. The missing amount of water was added.

### 3. Observation and measurements

After a period of 14 days, the surviving adults and the living juveniles per test vessel were extracted, applying a temperature gradient. For this purpose the content of each test vessel was carefully transferred to sieve vessels (mesh size approximately 0.8 mm). Each sieve vessel was put onto another vessel containing a fixing liquid. The vessels were positioned in MCFADYEN-Extractor. The

temperature was increased from approximately 25° to 40° C within two days. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* (adult, females and juveniles) were counted under a binocular.

### 4. Statistical analysis

Concerning the number of juveniles statistical analysis (Welch-t test for inhomogeneous variances one sided smaller,  $\alpha = 0.05$ ) revealed no significant differences between the control and treatment.

### RESULTS AND DISCUSSION

### A. Physical and Chemical Parameters

The soil-pH was 5.59 to 6.81. The water content was 43.85 to 47.98% of the maximum water holding capacity.

### **B.** Biological Findings

Observations on survival of adults and number of ite eniles are listed as follows. The results indicate that there is no significant difference in the number of juvenile between the control and the treatment group.

Table: Effects of AE F161089 on mortality, body weight change and reproduction of Hypoaspis aculeifer

Concentrate (mg a.i./kg dr	-		lt mortal of 8 repli	cates) /	,	mean nunder juveniles Standard deyî		Q (%	orgunction of control)	
control		A	16.3		, W	$264.0 \pm 54.5$			-	
100	% %		2193	0	Ŏ,	252.0 ± 30.	2 📞		95.5	

### C. Validity Criteria

Validity criteries Recommended by the (for control replicates) guideline	Obtained in this study
Mean adult female mortality & 60 00 00 000 000 000 000 000 000 000	16.3 %
mean number of juveniles per replicate (with 10 adult females introduced)	264.0
coefficient of variation calculated for the number of juvenile mites per coplicate.	20.6 %

All validity criteria were refet. Therefore this study is valid

### D. Test with toxic reference substance

Reference substance:
Date of most recent test:

Dimethoate

FEB 2010

Result:  $\mathcal{L} \subseteq \mathcal{L} \subseteq$ 

### E. Biological Endpoints Derived

 $LC_{50}$  (a failt mortality) > 100 mg p.m./kg soil dry weight NOF (reproduction) > 100 mg p.m./kg soil dry weight > 100 mg p.m./kg soil dry weight > 100 mg p.m./kg soil dry weight

### **CONCLUSION**

The chronic effect of 6-chloronicotinic acid (AE F161089) on predatory soil mites (*Hypoaspis aculeifer*) can be quantified as an overall-NOEC of  $\geq$  100 mg p.m./kg soil dry weight.

# IIA 8.14.1 Summary of preliminary data: biological activity & dose range finding Insecticidal activity

PECgw calculations indicate that the metabolite difluoroacetic acid might occur at concentrations of >0.1 μg/L in groundwater under certain worst-case conditions. Therefore, as part of studies required to demonstrate the non-relevance a screening test has been performed for the metabolite difluoroacetic acid (DFA) to compare its efficacy on target insects in comparison with the parent compound BYI 02960. The results are briefly summarized below.

Report:	KIIA 8.14.1/00; A. A. O. (2010)
Title:	Determination of the insecticidal efficacy of the metabolite datioroacotic acio
	(BCS-AA56716) compared to the parent compound BYI02960 (BCS-BZ89914)
Report No:	VAR 2010 01 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Document No:	M-386333-01-19
Guidelines:	No existing guideline "O" A " " " " " " " " " " " " " " " " "
Deviations:	Not applicable & & & & & & & & & & & & & & & & & & &
GLP:	Son-GIP & S

### EXECUTIVE SUMMARY

The insecticidal efficacy of BYI 192960 and its metabolise diffuoroacetic acid (BCS-AA56716; Purity: 95.8% w/w)) has been determined by loaf disc and whole plant screening systems. As expected, the parent compound BYI 02960 (technical; Purity: 96.2% w/w) efficiently controlled the relevant target insects

It could be demonstrated that the metabolite difluoroacetic acid does not have any insecticidal activity against any of the species tested, no ther in leaf disc nor in whose plant test systems.

### MATERIAL AND METHODS

### A. Materials

### Test material

Test item:

Diffluoroacetic acid (DFA, BCS-AA56716)

Type of test material:

Substance, technical (pure metabolite)

Origin Batch number: BCO 5984Q-1 (Batch code: BCS-AA56716-01-01)

Purity: 95.8% w/xy

2. Positive control

Test itekor. BYI 02960

Type of test material Substance, technical

Origin Batch number: 2009-000239 (Batch code: BYI02960-01-03)

Purity: 96.2% w/w
Stability of test compound: Not stated

### 3. Test organisms

Phaedon cochleariae PHAECO (Mustard beetle) A: Test organisms for the microtiter plate test: Myzus persicae MYZUPE (Green peach aphid)

Nilaparvata lugens NILALU (Brown plant hopper) Myzus persicae MYZUPE (Green peach aphid)

B: Test organisms for the Aphis gossypii APHIGO (Cotton aphid) greenhouse test:

Bemisia tabaci BEMITA (Silver leaf whitefly)

### B. Study design and methods

1. In-life dates Not stated in the report

### 2. Experimental treatments

For this comparing study, two different standardised test systems ha

### A - Microtiter plate testing:

A range of insects, covering relevant species that are known to be sensitive against BY 602960 mainly sucking insect segment), have been used in the actual study (mustard beetle, goen peach aphid and brown plant hopper). It is generally accepted that such microtiver systems are suitable to describe the intrinsic insecticidal potential of a tes Compound.

Methodology of the microtiter plate sest

### For Myzus and Phaedon (leaf coscs):

The cavities of 12-hole microtiter plates are filled up to half of its height with artificial insect diet (Phaedon) or agar gel (Myzus), respectively, and for each cavity a leaf disc (cabbage) of 2cm diameter is put on top.

In case of Myzus, the leaf discs are dread infected with all instars of Myzus at times of spraying. In case of Phaedow, two Rhaedow larvae are put together in each callity after spraying of the compounds.

The test compound BYI 02960 and its metabolite DFA (BCSAA5676), respectively, are dissolved in a mixture of water, acetorie, DNF and mulsifier (Emulsifier W), and four different concentrations are prepared. Each microfiler plate cavity is subsequently sprayed with one of the concentrations, resulting in application rates on the leaf discs of 500, 100, 20 and 4 g/ha. The leaf discs are allowed to dry afterwards for a certain period of time

The incubation time for the *Phaedon* tests are 7 days, for the *Myzus* tests 6 days before the test evaluation scarried out Efficacións girán in percentage of dead insects.

### For Nikaparvata (whole plant

A ca. 10 cm high cube is plugged into each covity of the microtiter plate, and small rice plants are grown in perlice in these tubes over one work.

Test compound solutions (solved)'s water, acetone, DMF, emulsifier W, RME1, AMS2) are prepared in concentrations at 0.8, £16, 0.052, 0.0064 ppm. These solutions are then sprayed into the tube, so that the spray is covering the small rice plants evenly.

The plants are infested with plant hoppers afterwards and stored for 7 days until the evaluation is done. Efficacy is given in percentage of dead insects.

### B - Greenhouse test:

BYI 02960 and its metabolite DFA (BCS-AA56716) were tested against a range of insects at application rates regarded as the relevant efficacy range for BYI02960 (70 - 100% efficacy).

Testing was done against the following relevant insect pests, all sensitive to BYI02960: Green peach aphid, Cotton aphid, Silver leaf whitefly.

### Methodology of the greenhouse test

### Spray test:

In each case, the parent compound and the metabolite, respectively, are dissolved in a mixture DMF (Dimethylformamide), emulsifier W, RME and AMS and are immediately applied by spraying a plant set, resulting in a complete coverage of the leaves with the test solution. At the the plants are already infested with the respective posects.

Incubation time for the Myzus and Aphis tests were 6 days and for the

### Drench test (only *Myzus*):

The respective compounds are dissolved in a mixture of water DMF smulsitier W.RME rape of methylester) and AMS (Ammonium surfate) and a specific volume 40 mc per 12 mL soil) of the solution is poured on the soil of a pour 7 cm chameter) in which a cabbage plant is growing. The plant is already infested with the respective insects at this time

Incubation time before the evaluation of the test is

Authorisation Procedure of Plant Protection Products (Draft 2003-09-19)

### RESULTS AND DISCUSSION

# A. Evaluation of results / Validity Criteria

Results of the test systems applied have been analyzed for fulfalling of EU goidance and German guidance:

- Metabolite must have clearly less than 50% of parent compound Efficacy of metabolite must be less than 30% of parent compound igher than 70%. EU guidanc@document efficacy

re (SANCO) of Sut <sup>7</sup> EU guida@e (SANCO/221/2000-rev.l0): Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances <sup>8</sup> Umweltbundesamt (UBA): Assessment of the Relevance of Metabolites in Groundwater in the Context of National



### **B.** Biological Findings

### A – Microtiter plate testing:

Insecticidal efficacy of BYI 02960 and its metabolite in microtiter plate system (referring to Table:

	Test object	MYZUPE	PHAEDON		Test object	NILALU, &
Microtiter plate	Test plant	Chinese cabbage	Chinese cabbage		Test plant	Rice
test	Application	Spray	Spray		Application	Spray W
	Evaluation	6 d	∡7 d	.6	Sevaluation &	7 d _Ç
	Conc. (g/ha)	Insecticidal	efficacy (%)	Ź,	Conc. (g/ba)	Efficacŷ (%)(
	500	100	💇 100 🥎	,	् <i>©</i> ँ 0,8 <sup>©</sup>	O' _@90
BY102960	100	100 🔬	©100 S	8	Ű "Q.¶6 "Ď	50
(BCS-BZ89914)	20	90 🔘 ້	0 0	J.	′ <b>%</b> ,032 💞	3,0
	4	70			0,006#	
	500					<b>√ 0 ₺</b>
Difluoroacetic acid (BCS-AA56716)	100				Ö,16	0
	20	& 0 °	~ 6 ~	J.	0,032	<b>7</b>
,	4		0	d	0 0064	° 0

While BYI 02960 shows significant efficacy up to 100% at the higher application rates, clearly no insecticidal efficacy at all is exhibited by the metabolite DFA (BCS-AA56 16). So Both, EU guidance and German guidance (UBA), are clearly fulfilled by these test in

the clearly fulffled by these test results obtained.

### B – Greenhouse efficaç

In greenhouse, the efficacy of BY 10296 and its metabolite has been determined in whole plant In greenhouse, the efficacy of BY 102960 and its metabolite has been determined in whole plant systems at concentrations between 20 and 0.032 pppp, regarded as the relevant efficacy range for BYI 02960 (70 - 100% efficacy).

Results are summarized in the following table:

Efficacy of BYI 02960 and its metabolite in greenhouse test on plants (referring to B)

	Test object	MANAGER					
	. oot object	MYZUPE	APHIGO	BEMITA		Test object	MYZUPE
Whole plant spray test	Test plant	Bell pepper	Cotton	Cotton		Æst plant	Cabbage
	Application	Spray	Spray	Spray	✓	Application 🛴	Dr <b>e</b> nch "
	Evaluation	6 d	6 d	ੴ 8 d	Ÿ	Evaluation	₹ d ₹
(	Conc. (ppm)	Ins	ecticidal efficacy	<b>%</b> )	(J)	Conc. (pp	2 4
	20		Ş	98 🛴		28	
DVIOCOC	4	100	100	98 🔎	Po		<b>000</b> ©
BYI02960 (BCS-BZ89914)	0,8	75	<b>95</b> 0° ″	<b>~</b> € ′ @		Q0,8 \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	& 75 D
(======================================	0.16	0		<b>00</b>		_@ 0.1 <b>6</b>	0,5
	0.032	0	~ 0 @		_ (	0.6\$2	y (0°
	20				Ö	20	
Difluoroacetic	4	0 🗶				4 , .	© 0
acid	0,8	0	, N 0 0	, 4 <sup>λ</sup> ο 9 <sup>λ</sup> ,	~		
(BCS-AA56716)	0.16	0 🗸	( O )	0 0	$\mathbb{Q}^{'}$	<b>1</b> 6	<b>"</b>
	0.032				) }	0.032	√ Q 0

The results presented in the table above demonstrate very clearly that the metabolite DIX (BCS-AA56716) does not show any insecricidal dicacy at all, in contrast to the parent compound BYI 02960.

### **CONCLUSION**

Summarising the results of the two test systems, the following can be stated

In all test systems it could be definoustrated in a very uniform manner that the metabolite difluoroacetic acid (BCS-AA \$6716) Goes not exhibit any substantial insecticidal efficacy in comparison to the parent compound BMI 02960.

# Assessment of relevance to potential impact on non-target species

Risk assessments for all non-targer species are performed in product specific Annex III dossiers.

Effects on biological methods for sewage treatment **IIA 8.15** 

Report:	KII 8.15/00; (2010)
Report:	Activated Sludge, Respiration Inhibition Test with BYI 02960 (tech.)
Report No:	<b>20</b> 10/0089/01
Document No:	M-377 1-01-1
Guidelines:	EC to. 440/2008 anethod C.11 (2008) QCCD 200 (1984)
	QCCD 209 (1984) V
Deviations:	None , , , , ,
GLP:	Yes (certified laboratory)
GLP: F	

### **EXECUTIVE SUMMARY**

The study was performed to assess the toxicity of BYI 02960 (tech.) to bacteria by measuring the respiration rate.

Activated sludge was exposed to BYI 02960 (Origin Batch No: 2009-000239; Batch code: BYI 02960-01-03; TOX 08508-00; purity 96.2% w/w) at nominal concentrations of 100, 180, 320, 560 and 1000 mg a.i./L, respectively. The respiration rate of each mixture was determined after aeration periods of 3 hours. A reference compound (3,5-Dichlorophenol) was tested at concentrations of 25 and 40 mg/L, respectively.

After an incubation period of 3 hours, BYI 02960 (tech) showed 23.3% respiration inhibition of activated sludge at the highest test item concentration of 1000 mg aQ/L.

Hence, the EC<sub>50</sub> was higher than 1000 mg a.i./L. The EC<sub>10</sub> was determined to be a MATERIAL AND METHODS

A. Materials

1. Test material

### 1. Test material

Test item: Substance, Cchnicat Type of test material: Berge powder & Chemical state and description: ©102000@22313 Specification no:

2009-000239 (Batch code: BYI 02960-01-03)
TEX 08508-00 Origin Batch number:

Sample description

2(5H)-furanon 4-[[(6%) hloro 3-pyridinyi) moth CAS name:

difluonoethylomino

CAS#: 951659-40-8

1)(2,2-difluoroethyl)amino]furan-2(5H)-one 40 6-cht Topyridin-3 IUPAC name:

Purity:

Purity: Stability of test compound

# 2. Test organism and s

Mixed population of aquatic microorganisms Type:

(activated Sludge)

Reration tank of a domestic sewage treatment Origin:

plant Municipal STR Cologne-Stammheim)

Date of collection: 14 June 2010

A vration of the activate  $\mathcal{O}$  ludge at 20°C ± 2°C, Storage:

daily fed with synthetic medium

Synthetic sewage feed 16.0 g peptone

(per 11 water): 1 Lorg meal extrac

g KOTPO<sub>4</sub>

### B. Study design and methods

1. In life dates June 14 to 21, 2010

### 2. Design of biological test

10 mL of sludge suspension were dried in order to calculate the amount of sludge suspension to whieve a concentration of activated sludge of 2 g/L (dry weight) suspended solids. The valuated amount of sludge suspension was taken and filled up to a defined end volume with deionised water. The pl measured and adjusted to pH 6-8.

8 mL of the synthetic medium and 100 mL of activated studge were added to the dissolved test item. The mixture was filled up with deionised water to 250  $\mathrm{pL}$  and aerated at 20°C ± 2°C.

The exposure medium with the reference substance was prepared by adding 8 mb of the synthetic medium, 100 mL of activated sludge and a defined amount of the stock solution to achieve the test concentrations and was filled up to 250 mL with deionsed water.

A control at test start and end (control 1, 2) was tested in the same way. The exposure medium to determine the physico-chemical oxygen consumption was prepared as well as the medium, with the test item, but without activated sludge. To measure the oxygen consumption, 250 mL of sludge with Pest item (or control or reference compound) was incubated for h in 300 mp closed Erlenbieyer Pasks.

### 3. Observation and measurements

Oxygen consumption was measured and recorded after an abration me of 3 hours. Thereafter, temperature and pH of the exposure medium were measured. For measurement, the content of the Erlenmeyer flasks was completely transferred to 250 mlk BOD bottles and 02-content was measured with an O<sub>2</sub>-meter (redox electrode).

The respiration rate for each concentration was determined graphically from the linear part of the curve The pH was adjusted to 7±05 with HCl.

B. Biological Findings

After an incubation period of 3 hours, analysis of the respiration rates (by probit analysis) gave the following values: of O2-content versor time. The inhibitory effect of the vest item at a particular concentration is

Table: Effects of BYI 02960 (tech.) on respiration rate of activated sludge

Test item concentration (nominal) [mg/L]	Respiratory rate test item [mg/L x h]	Physchem. O <sub>2</sub> consumption [mg/L x h]	Respiratory rate - phys - chem O <sub>2</sub> consumption [mg/L x h]	Inhibition [%]
100	26.6	0.0	26.6	0.0
180	26.0	0.0	26.0	0.0
320	25.7	0.0	25.7	0.05
560	23.3	0.0	23.3	\$16 23.3. \(\frac{1}{23}\)
1000	19.5	0.0*	19.5 W	23.3 V V
Control mean	25.4	e	- 8	
Control 1	24.9	- 0	A,	
Control 2	26.0	- 🛋	Q B°	

Comments:

Test concentrations are given as nominal concentrations and were not confirmed by malytic methods.

Table: Selected effective concentrations (ECX) of BY 02960 (techn) and their 95% and 99%-confidence limits (according to Fieller's theorem)

Parameter	EC10 @ .	E@20 \$ 0	E650 0 %
Value [mg a.i./L]	472.5	015.2	Ad. Q
lower 95%-cl	259.9	\$\P595.\%\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	n.d.
upper 95%-cl	<b>963</b> .9	5945.3 ,	n.d. y
lower 99%-cl	150.3	237.8 0 0	ng.d.
upper 99%-cl	1666	15545.8	@.d. ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~

n.d.: not determined due to mathematical reasons or imappropriete data

Computation of variances and confidence limits was adjusted to metric data.

Slope function afte Litchfield and Wilcoxon: 5.68

1984).

### C. Validity Griteria

All validito criteria of the test to thou were met:

- respiratory rates of the 2 controls differed less than 15% from each other
- the EC<sub>50</sub> of the reference compound 35-Dickbrophenol was in the range 5-30 mg/L

### D. Test with toxic reference substance

A reference test with 3,5-Dichlorophenol hower an Eco of 8.7 mg/L (95% CI: 5.4 – 13.9 mg/L).

### E. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

EC50: >1000 mg/a.

EC<sub>10</sub>: 472.5 mg/L (95% CI: 29.9 - 963.9 mg/L)

### CONCLUSION

BYI 02060 (tech.) showed \$3.3% respiration inhibition of activated sludge at the highest test item concentration of 1000 mg/L. Thus, the EC<sub>50</sub> is > 1000 mg a.i./L.

<sup>\*</sup> The physico-chemical oxygen Consumption has been determined at 1000 mg/L test item concentration. As no physico-chemical oxygen consumption was observed at that test term concentration this observation also holds true for the lower test item concentrations.

IIA 8.16.1 Other/special studies - laboratory studies

Effects on honey bees

In this section all studies in honey bees, which do not have specific dossier point headings, are summarized, this includes 10 day feeding studies on BYI 02960 and plant and environmental matabolites as well as a larvae feeding study.

Report:	KIIA 8.16.1/01; 2010)
Title:	BY102960 – Assessment of Chronic Effects to the Honey Dee, Apis mellifera L.,
	in a 10 Days Laboratory Fedding Test 🔪 🧳 🗘 🐧 👸
Report No:	S10-02924
Document No:	M-400539-01-2
<b>Guidelines:</b>	No specific guideline available
<b>Deviations:</b>	Not applicable \( \frac{1}{2} \) \( \frac{1} \) \( \frac{1}{2} \) \( \frac{1}{2} \)
GLP:	Yes (certified laboratory)

### **EXECUTIVE SUMMARY**

The chronic effects of the test item BYI 02960 Batch ode: BO 02960 96.2% w/w) on the honey bee, Apis mettifera L., in a 10 days continuous feeding test in the laboratory were assessed.

Over a period of 10 days, honey bees were exposed to 50% (NV) sucrose solution containing nominally 100, 300, 1000, 3000 and 10000 µg a.i./Lof the test item BY 102960 by continuous and ad libitum feeding. All test item feeding solutions contained additionally 1% acetone. The control group was exposed for the same period of time under identical exposure conditions to an untreated 50% (w/v) sucrose feeding solution also containing 1 // acetore Mortality, while that effects and behavioural observations were assessed every day throughout the 10 days exposure period.

Samples and retain samples of all feeding solutions and the spock solution were taken for analysis.

The accumulated nominal intake of the test frem BYI 02960 via BYI 02960-treated sucrose solution was 0.04, 0.13, 0.48 1.49 and 4.60 µg a 17 bee, respectively, after 10 days of continuous exposure.

It can be concluded that the continuous feeding of boney bees in the laboratory over a period of 10 consecutive days with the test item BYI 02960 at treatment levels of 100, 300, 1000, 3000 and 10000 μg a L caused no adverse effective garding mortality, sublethal effects and behaviour. Since at all other cose levels, higher and lower than 300 µg a.l./L, the food consumption was not statistically significantly different when compared to the food consumption of the control group, the statistically significantly reduced food consumption at the second highest dose level of 300 μg a.i./L should not be overestimated.

The NOEC (No Observed Effect Concentration) was determined to be 10000 µg a.i./L at the end of the test period

### MATERIAL AND METHODS

### A. Materials

1. Test material

Test item:

Type of test material:

Chemical state and description

Specification No.:

BYI 02960

Substance, technical

Beige powder

102000022313

Origin. Batch No.: 2009-000239 (Batch code: BYI 0296001-03)
Sample description: TOX 08508-00 (Text item code: 2000-01178)
CAS name: 2(5H)-Furanone, 4-[[(6-chloro-3-poridinyl)meth)]

| difuoroethyl)appino]
| CAS#: 951659-40-8 |
| Purity: 96.2% w/w fanalytical)

IUPAC name: 4-[(6-chloropyridin-3-ylmethyl)(2/,2-diffooroethyl)aminoffurap

(5H)<sub>7</sub>ome

Stability: Expiry date: 46.01.2011, when stored at +25 ± 5°C

Treatment 50 % (w/v) sucrose solution, containing the test item BYI 02960

at the nominal concentration levels of 100, 300, 1000, 3000 and

10000 µg a iQL as well as 1% acetone

Control 50% (w/v) aqueous sucros solution containing 1% acettore

### 2. Test organisms

Species:
Common name:

April meltiplera L.

Ploney See

Age or developmental stage at test start: Adult work bee

### B. Study design and methods

1 In life dates V S INV 21 to 31 2014

### 2. Experimental treatments

A study was conducted to determine the chronic effects of the test item BYI 02960 on the honey bee, *Apis mellifera* L., in \$10 days continuous feeding test in the laboratory.

The honey bees were continuously and ad libitum feet with 50% (w/v) sucrose solution, containing the test item BYL05960 at the nominal concentration levels of 100, 300, 1000, 3000 and 10000  $\mu g$  a.i./L as well as 1% acctone, respectively on the control group the honey bees received an untreated 50% (w/v) aqueous sucrose solution containing 1% acctone, and bitum.

The feeding solutions were offered ad libitum to each cage of 10 bees in plastic syringes (Germany). The rip of each syringe was removed so that the bees had access to the feeding solution of very morning the syringes of all test cages (i.e. test item and control) were replaced by new syringes, filled with freshly prepared feeding solution. The weight of the syringes was determined before and after reeding on the next day in order to determine the mean food consumption of the bees per 10st cage.

During the entire test period, the bees were kept in cages made of stainless steel (base: 8.2 cm x 4 cm; height: 6 cm). The front side of the cages were equipped with a transparent pane so that the bees could be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test animals. The test cages are lined with filter paper.

The feeding solutions were offered to the bees in syringes fitted into the test cages.

The study was carried out with the following treatments:

- five doses of the test item BYI 02960
- the control

At each test item treatment level, 10 replicates of 10 honey bees each were tested (i.e. in total 100 honey bees per test item concentration). The control group comprised 30 replicates of 10 km by each (i.e. in total 300 honey bees). A toxic standard was not included in this test, since a toxic reference substance had not been defined or validated for this type of study.

3. Observation and measurements

### 3. Observation and measurements

### Mortality:

day at about the same time of The number of dead bees in the individual test cages was recorded every day during the 10 days test period.

sed according to the followings Abnormal behavioural effects and behavioural different categories and recorded in the raw data:

- affected (bees still up ght and attempting to a =
- apathy (bees show only fow or delayed reactions to stimulation [Se. light, air blow] ap =bees are either sitting motionless in the cage or walk but not correctly)
- moribund Dees cannot walk and show only very feeble movements of legs and m =antennae, only weak responsoto stimulation, beekmay recover but usually die)

The mortality [%] in the test item treatment was calculated from the number of dead bees and the total number of introduced begon the test item treatment group. The mortality of the test item treatment was corrected for corresponding control mortality, according to the formula of ABBOTT (1925), modified by SCHNEIDER-ORELLI (\$947).×

Corrected morphity 
$$M = \frac{a - c}{100 + c} \times 100$$

Corrected mortality (%) M

Mortality in the test item group (2)

Mortalit in the Sontrol Foup

### Food consumption:

The consumption of feeding solution per beg day was calculated by dividing the total daily consumption per cage by the mean number of living bees at the corresponding time interval. The mean value per treatment group as well as the daily mean food consumption per bee was calculated.

test item per beg was calculated as follows: The mean intake of

### Statistical evaluations:

Fisher's Exact Test (Bonferroni-Holms corrected, one-sided,  $p \le 0.05$ ) was used to evaluate whether there are significant differences between the mortality data of the test item treatment group and the control group.

For the statistical comparison of the food consumption, non-rounded values were taken. Data of food consumption were tested for normality using Shapiro-Wilks' test. If normality was observed, the trest (one-sided,  $p \le 0.05$ ) was applied. If the data deviated from the normal distribution, statistical analysis was performed by using the Mann Whitney – U test. Statistical calculations were made by using the statistical program SAS release Version 9.2.

### Residue analysis:

The residue analysis of the feeding solutions was performed in an independent study by , the analytical attached as an integral part of the study report CropScience AG,

### Storage and shipment of samples:

Samples taken of the feeding solutions and the stock solution were stored deep frozen (≤ √18°C) in the

Campo H lassorator within 30 minutes the samples were shipped on dry ice from to the residue analysis laboratory of the sponsor Bayer Crop Science

### RESULTS AND DISCUSSION

### A. Environmental Parameters

summarized as follo Measurements of climatic parameters during the test are

Test temperature: Relative air humidit Light intensity:

50 58 % Darkness sxcept daring sissessmeat

# B. Verification of the Application Volume

The residue analysis of the feeding solutions was performed in an independent study (analytical report is part of the study report, see Appendix 3)

### C. Biological Findings

The results of the 10 days feeding test with ocontrol group fed with untreated sucrose solution Tot.

In treatment stone and diluted. containing 1% acetone and the test item treatments with 200, 300, 1000, 3000 and 10000 µg a.i. /L of BYI 02960 First dissolved in accione and diluted in 50% sucrose solution are presented in the table below.

### **Table** Mean consumption of feeding solution, mean intake of test item accumulated over all test days and cumulative mortality at the final assessment on day 10

		Test Item				
Treatment Level <sup>1</sup>	Control	100	300	1000	3000	10000
				[µg a.i./L]	>	
Mean consumption of feeding solution [mg/bee] <sup>2</sup>	57.4	52.4	50.8**	56.4	\$58.8	54.9
Mean intake accumulated over test days [µg a.i./bee]	-	0.04	0.13	0.48	1.49	74.64 7 7 7 7 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Cumulative mortality [%]	18.0	9.0	14.0	Q.0	24.00	100*
Corrected cumulative mortality [%]	-	-1 <del>1.0</del> 0	-4.9	₹ -8. <b>5</b> , °	\$\frac{1}{2}\text{.3}	4 2 2 2 1 24 2
The control group was fed with untreated 50% (w/v) sucrose solution in the dwith 1% asctone; all test						

- The control group was fed with untreated 50% (w/v) sucrose sometion mixed with 1 % accione; afficest item treatment groups were fed with BYI 02960-treated 50% (w/v) sperose solution named with 1%
- The mean values per cage over the test period were used as basis for the calculation of the wean consumption of feeding solution per the atment over the test period
- Determined to be the NOEC (Fisher's Exact Pest (Boyfferront Holms Forrected one-sided,  $p \le 0.05$ )) Significantly reduced compared to the control group (t-test with Botherroni Holms Forested).  $p \le 0.05$ )

### D. Validity Criteria

Not applicable. No guideline is available for the

E. Biological Endpoints Derived After 10 days of continuous exposure mortality at all test item treatment levels was not significantly increased compared to the control group.

After 10 days of exposure the cumulative control mornality accounted for 18.0%, as determined at the final assessment

Furthermore on all test item treatment levels, no remarkable sublethal effects or behavioural abnormalities were observed throughout the entire test period. The highest test item treatment level of 10000 µg a.i./L was determined to be the NOEC (No Observed Exfect Concentration; Fisher's Exact Test; Bonferroni-Holms corrected one-sided, p. 0.050

At the 300 µg a.i/L test tem treatment level the mean food consumption per bee was slightly, although statistically significantly reduced when compared to the food consumption of the control group (t-Test with Bonferioni-Holms correction, one sided, 15% 0.05%. However, at all other dose levels, higher and lower than 300 µg a.i./L. i.e. 100, 1000, 3000 and 10000 µg a.i./L), the food consumption was not statistically significantly different when compared to the food consumption of the control group.

The accumulated nominal intake of the test item BYI 02960 via BYI 02960-treated sucrose solution 149 and 4.64 µg a.i./be after 10 days of continuous exposure. was 0.04, 0.13, 0.48

From the sults desented above the following biological endpoints can be derived:

NOEC 10000 µg a.i./L

### **CONCLUSION**

It can be concluded that the continuous feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item BYI 02960 at treatment levels of 100, 300, 1000, 3000 and 10000 µg a.i./L caused no adverse effect regarding mortality, sublethal effects and behaviour. Since at all other dose levels, higher and lower than 300 µg a.i./L, the food consumption was not statistically significantly different when compared to the food consumption of the control group, the statistically significantly reduced food consumption at the second highest dose level of 300 µg a.i./L should not be overestimated.

The NOEC (No Observed Effect Concentration) was determined to be 70000 µg a.i./L at the end of the test period.

Report:	KIIA 8.16.1/02;
Title:	BYI 02960-difluoroethyl-arnino-fyranone (BYI 02960-DYAF) Assessment of Chronic Effects to the Lioney Boo, Apis welliferal, in a 10 Days Continuous
	Chronic Effects to the Honey Bee, Apis wellifer QL., in a 10 Days Continuous
	Laboratory Feeding Laprit Test
Report No:	S11-01959 Q
Document No:	M-425174-01-2 Q
<b>Guidelines:</b>	No specific guideline available
<b>Deviations:</b>	Not applicable & & & & & & & & & & & & & & & & & & &
GLP:	Yes (certified laboratory)

### **EXECUTIVE SUMMARY**

The chronic effects of the test item BYL 02960 DFE A (Origin Batch No: NLL 86/1-12-1; Batch code: BCS-CC98193-01-03 POX 09255-09, Purity 98.9% w/w) on the honey bee, Apis mellifera L., in a 10 days continuous feeding limit test in the laboratory were assessed.

Over a period of 10 days, honey bees were exposed to 50% (w/v) aqueous sucrose feeding solution, containing nominally 10000 ug a.i./L of the test item BY 02966 DFEAF by continuous and *ad libitum* feeding. Because the test item was first dissolved in accione and then diluted with aqueous sucrose solution, the final test item feeding solution contained 1% accione. The control group was exposed for the same period of time under identical exposure conditions to untreated 50% (w/v) aqueous sucrose feeding solution, also containing 0% accione. Mortality, subjethal effects and behavioural observations were assessed every day throughout the 10 days exposure period.

The accumulated normal intake of the test item BYI 02960-DFEAF via BYI 02960-DFEAF - treated aqueous sucrose feeding solution was 455 µg a 1./bec after 10 days of continuous exposure.

Samples and retain samples of all feeding solution and the stock solution were taken for chemical analysis.

It can be concluded that the continuous feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item BYI 02960-DFEAF at the treatment level of 10000  $\mu$ g a.i./L caused no adverse effect regarding mortality, sublethal effects and behaviour. As the overall mean daily food uptake (i.e. the ascrage value over 10 days) in the test item treatment group was virtually identical to the untreated control group and because only on one single day during the 10 day continuous exposure period the mean food consumption per bee was significantly lower in the test item treatment group compared to the control group, it can be concluded that there was no repellent effect of the test item at the treatment level of 10000  $\mu$ g a.i./L.

The NOEC (No Observed Effect Concentration) was determined to be 10000 µg a.i./L at the end of the test period.

#### MATERIAL AND METHODS

#### A. Materials

### 1. Test material

Test item: BYI 02960-difluoroethyl-amino-furatione

(BYI 02960-DFEAF; BCS-CC981🔊)

Pure metabolite Type of test material: Chemical state and description Powder, white

NLL 8671-12∰ (Batch coder BCS-CC9819 Origin Batch No.:

Sample description:

CAS#: Purity:

Stability of test compound:

nominally 10000 µg and L of the test item BYL02960-DFEAG as well as 1% acetone 50% (W/V) Treatment

Control

### 2. Test organisms

Species:

Common name:

Age or developmental stage at test

# B. Study design and methods

June 7

A study was conducted to determine the chronic effects of the test tem BYI 02960-DFEAF on the honey bee, Apis metGera L. in a 10 days Continuous feeding limit test in the laboratory. The NOEC value (No Observed Effect Concentration) for mortality was determined at the end of the test period.

The honey bees were ged continuously and ad libitum with a 50% (w/v) aqueous sucrose solution, containing the test item BY 02960-DFEAF at the nonanal concentration level of 10000 µg a.i./L as well as 1% acetone. For the proparation of the test item treatment level, the active substance content of the test item BCS-CC 193 BYI 02960-DEAF) was considered to be 100% and not corrected for the analysed content of 98.9% (w/w).

In the control group the honey bees received an untreated 50% (w/v) aqueous sucrose solution containing 1% aceton, ad libitum.

The feeding solutions were offered ad libitum to each cage of 10 bees in plastic syringes (

Germany). The tip of each syringe was removed so that the bees had access to the feeding solution. Every morning the syringes of all test cages (i.e. test item and control) were replaced by new syringes, filled with freshly prepared feeding solution. The weight of the syringes was determined before and after feeding on the next day in order to determine the mean food consumption of the bees per test cage.



During the entire test period, the bees were kept in cages made of stainless steel (base: 8.2 cm x 4 cm; height: 6 cm). The front side of the cages were equipped with a transparent pane so that the bees could be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test animals. The test cages are lined with filter paper.

The feeding solutions were offered to the bees in syringes fitted into the test cages.

The study was carried out with the following treatments:

- one concentration of the test item BYI 02960-DFEAF (difluorgethyl-amino-faranons)
- the control

The test item treatment was tested with 10 replicates (cages), each containing 10 bees (i.e., in total 100 honey bees). The control group comprised 30 replicates of 10 bees each (i.e., in total 300 honey bees).

A toxic standard was not included in this test, since a foxic reference substance had not been defined or validated for this type of study.

# 3. Observation and measurements

### Mortality:

The number of dead bees in the individual test cases was recorded every day at about the same time of day during the 10 days test period.

Abnormal behavioural effects and behavioural differences were assessed according to the following categories and recorded in the raw data:

- a = affected (bees will uproth and attempting to walk but showing spens of reduced coordination)
- ap = apathy (bees show only low or delayed reactions to stimulation [i.e. light, air blow]

  bees are either sitting notioness in the cage or walk but not correctly)
- m = moribund (bees cannot walk and show only very feel to movements of legs and antennae only weak response to stimulation bees may recover but usually die)

The mortality [%] in the test item reatment was calculated from the number of dead bees and the total number of introduced bees in the test item treatment group. The mortality of the test item treatment was corrected for corresponding control mortality, according to the formula of ABBOTT (1925), modified by SCHNEIDER ORELLI (1947).

Corrected in relative 
$$M = \frac{t - c}{100 - c} \times 100$$

 $M = \text{Corrected mortality}(\%)_{Q}$ 

= Mortality in the est item group %

= OMortality in the control group (%)

#### Food consumption

The consumption of feeding solution per bee/day was calculated by dividing the total daily consumption per cage by the mean number of living bees at the corresponding time interval. The mean value per reatment group as well as the daily mean food consumption per bee was calculated.

The mean intake of test item per bee was calculated as follows:

		Mean consumption of			
Mean intake of test item [μg a.i./bee]	=	feeding solution [mg/bee]  Density of the sucrose solution [g/cm³]	X	Concentration of feedin [µg a.i./µL]	ng solution of the solution of

#### Statistical evaluations:

Fisher's Exact Test (Bonferroni-Holms corrected, one-sided,  $p \le 0.05$ ) was used to evaluate whether there are significant differences between the mortality data of the test item treatment group and the control group.

For the statistical comparison of the food consumption, non-rounded values were taken. Data of food consumption were tested for normality using Shapiro-Wilks, test. It normality was observed, the t-Test. (one-sided,  $p \le 0.05$ ) was applied. If the data deviated from the normal distribution, statistical analysis was performed by using the Mann Whitney – U test. Statistical calculations were made by using the statistical program SAS release Version 9.2.

# Residue analysis:

The residue analysis of the feeding solutions was performed in an integral part of the study report.

Storage and shipment of samples

Samples taken of the feeding solution and the stock folution were stored deep frozen ( $\leq -18^{\circ}$ C) in the most constant of the samples were shipped on dry ice from the stock form the samples were shipped analysis laboratory of the ponson Bayer Crop Science (AG).

#### RESULTS AND DISCUSSION

#### A. Envirónmental Parameters

Measurements of simatics parameters during the test are summarized as follows:

Test temperature

Relative air http://doi.org/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.1000/10.100/10.100/10.100/10.100/10.100/10.100/10.100/10.1

# B. Verification of the Application Volume

The residue analysis of the feeding solutions was performed in an independent study (analytical report is part of the study report).

# C. Biological Findings

The result of the 0 days feeding test with a control group fed with untreated sucrose solution containing 1% seetone and the test item treatment of 10000  $\mu g$  a.i. /L of BYI 02960-DFEAF, first dissolved in acctone and diluted in 50% sucrose solution are presented in the table below.

#### **Table** Mean consumption of feeding solution, mean intake of test item accumulated over all test days and cumulative mortality at the final assessment on day 10

Treatment Level <sup>1</sup>	Control	Test Item 10000 μg a.i./L
Overall mean daily consumption of aqueous sucrose feeding solution [mg/bee] <sup>2</sup>	51.4	51.5
Mean intake accumulated over test days [μg a.i./bee]	-	4.35
Cumulative mortality [%]	3.67	0.00
Corrected cumulative mortality [%]	- &	-3,8,1

The control group was fed with untreated 50% (w/v) aqueous sperose feeding colution mixed with 1% acetope the test item treatment group was fed with 50% (w/v) aqueous sucrose feeding solution containing BST 02960-

# D. Validity Criteria

Not applicable. No guideline is available for this type of

# E. Biological Endpoints Derived

After 10 days of continuous exposure, mortality in the test item treatment group was not significantly different compared to the control group. The cumulative control portality accounted to 3.67%, as determined at the final assessment (ay 10). In the test item treatment group at 1000@ug a.i./L, the cumulative mortality at the final assessment (day 10) accounted to 0.00% (corrected -3.81 %).

Furthermore, neither subjethal effects nor behavioural abnormalities were observed throughout the entire testing period in the test item treatment group. The test item treatment level of 10000 µg a.i./L was determined to be the NOEC(No Observed Effect Concentration, Fisher's Exact Test; (Bonferroni-Holms corrected one-sided, po≤ 0.05®

The overall mean daily consumption of the aqueous sucrose feeding solution (i.e. the average value over 10 days) in the test them the atment group was virtually dentical to the untreated control group (51.5 mg/bee in the test item treatment compared to 51.4 mg/bee in the control group).

The mean daily consumption of the aqueous sucrose feeding solution was statistically significantly reduced in the test item reatment group (42 mg/bee) compared to the control group (49 mg/bee) only on day 9, (t-test, one-sided  $0 \le 0.05$ ). On all other test days, the mean daily consumption of the aqueous sucrose feeding solution was affinost identical in the test item treatment group compared to the control group (day-by-day comparison).

The accumulated nominal intake of the test item BYI 02960-DFEAF via BYI 02960-DFEAF - treated aqueous sucrose feeding solution was \$35 μg/a.i./bee after 10 days of continuous exposure.

presented above the following biological endpoints can be derived:

10000 μg a.i./L

It can be concluded that the continuous feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item BYI 02960-DFEAF at the treatment level of 10000 µg a.i./L caused

<sup>&</sup>lt;sup>2</sup> The mean values per cage over the test period were used as basis for the calculation of the overall mean daily consumption of the aqueous sucrose feeding solution per freatment over the test period.

<sup>\*</sup> Determined to be the NOEC (not significantly different compared to the control, Figure's Exact Test, Bonferroni-Holms corrected, one-sided,  $p \le 0.05$ )

no adverse effect regarding mortality, sublethal effects and behaviour. As the overall mean daily food uptake (i.e. the average value over 10 days) in the test item treatment group was virtually identical to the untreated control group and because only on one single day during the 10 day continuous exposure period the mean food consumption per bee was significantly lower in the test item treatment group compared to the control group, it can be concluded that there was no repellent effect of the test trem at the treatment level of  $10000 \mu g \ a.i./L$ .

the treatment level of 10000 μg a.i./L.

The NOEC (No Observed Effect Concentration) was determined to be 10000 μg a.i./L at the end of the test period.

Report:	KIIA 8.16.1/03; (2012)
Title:	BYI 02960-hydroxy – Assessment of Chronic Effects to the Honey Be Apis inclifer W
	L., in a 10 Days Continuous Laboratory Feeding Limit Test @ S
Report No:	S11-01960
Document No:	M-425212-01-2
<b>Guidelines:</b>	No specific guideline available
<b>Deviations:</b>	Not applicable A A A A A A A A A A A A A A A A A A A
GLP:	Yes (certified laboratory) & y & D O

#### **EXECUTIVE SUMMARY**

The chronic effects of the test item BY102960-hydroxy (Origin Batch No: SES 19215-710; Batch code: BCS-CQ74364-PU-01; Purity 95.5% w/w) on the honey bee, Apis mellifera L. in a 10 days continuous feeding limit test in the laboratory were assessed.

Over a period of 10 days, honey bees, were exposed to 50% (w/v) aqueous sucrose feeding solution, containing nominally 0000 µg a.i.d. of the test item BY 02960-hydroxy by continuous and *ad libitum* feeding. Because the test item was first dissolved in actions and then diluted with aqueous sucrose solution, the final test item feeding solution contained 1% acetons. The control group was exposed for the same period of time under identical exposure conditions to untreated 50% (w/v) aqueous sucrose feeding solution, also containing 1% acetone. Mortality, sublethal effects and behavioural observations were assessed every day throughout the 10 days exposure period.

Samples and retain samples of all feeding solutions and the stock solution were taken for chemical analysis.

The accumulated normal intake of the test from BYI 02960-hydroxy via BYI 02960-hydroxy - treated aqueous sugrose feeding solution was 4.28 µg ay bee offer 10 days of continuous exposure.

It can be concluded that the continuous feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item by I 02060-horroxy at the treatment level of 10000 µg a.i./L caused no adverse effect regarding mortality, subjethal effects and behaviour. As the overall mean daily food uptake (i.e. the average value over 10 days) in the test item treatment group was almost identical to the untreated control group and because on every single day during the 10 day continuous exposure period the mean food consumption per bee was not significantly lower in the test item treatment group compared to the control group at can be concluded that there was no repellent effect of the test item at the treatment evel of 10000 µg a.i./L.

The NOEC No Observed Effect Concentration) was determined to be 10000 µg a.i./L at the end of the test period.

#### MATERIAL AND METHODS

#### A. Materials

# 1. Test material

BYI 02960-hydroxy (BCS-CQ74364) Test item:

Type of test material: Pure metabolite Chemical state and description Solid, brown

SES 11215-7-10 (Batch code: BCS-Q74364-PU-0 Origin Batch No.: Not available (Test item code: 201 001604) Sample description:

CAS#: Not available

Purity: 95.5% w/w

Stability of test compound: Expiry date: 4 12.2012, when stored at +5

50% (w// Faqueous sucrose feeding solution, containing) Treatment

nominally 10000 μg a GL of the test icom BY 02960 Hydroxy

Control

#### 2. Test organisms

Species:

Common name:

Age or developmental stage at test

# B. Study design and methods

1. In life dates

#### 2. Experimental treatments

A study was conducted to determine the chronic effects of the test item BYI 02960-hydroxy on the honey bee, Apis mellifera L., In a 10 days continuous feeling limit test in the laboratory. The NOEC value (No Observed Effect Concentration) for mortality was determined at the end of the test period.

The hone bees were fed continuously and ad libitum with a 50% (w/v) aqueous sucrose solution, containing the test item BYI 02960 hydroxy at the nononal concentration level of 10000 µg a.i./L as well as 1% acetone. For the preparation of the test item treatment level, the active substance content of the test item B6S-CQ74364 (BYI 02960-hydroxy) was considered to be 100% and not corrected for the analysed content of 95.5% ©w/w

In the control group the boney sees received in untroated 50% (w/v) aqueous sucrose solution containing 1% acetone, ad libitum.

The feeding solutions were offered ad libitum to each cage of 10 bees in plastic syringes (

Germany). The tip of each syringe was removed so that the bees had access to the ceding solution. Every morning the syringes of all test cages (i.e. test item and control) were replaced by new symples. Filled with freshly prepared feeding solution. The weight of the syringes was determined before and after feeding on the next day in order to determine the mean food consumption of the bees per test cage.

During the entire test period, the bees were kept in cages made of stainless steel (base: 8.2 cm x 4 cm; height: 6 cm). The front side of the cages were equipped with a transparent pane so that the bees could

be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test animals. The test cages are lined with filter paper.

The feeding solutions were offered to the bees in syringes fitted into the test cages.

The study was carried out with the following treatments:

- one concentration of the test item BYI 02960-hydroxy
- the control

The test item treatment was tested with 10 replicates (cages) each containing 10 bees (i.e. in total 100 honey bees). The control group comprised 30 replicates of 10 bees each (i.e. in total 200 honey bees).

A toxic standard was not included in this test, since a wice reference substance had not been defined or validated for this type of study.

# 3. Observation and measurements

# Mortality:

The number of dead bees in the individual test cases was recorded every day of about the same time of day during the 10 days test period.

Abnormal behavioural effects and behavioural differences were assessed according to the following categories and recorded in the raw data:

- a = affected (bees still upright and attempting to walk but showing signs of reduced coordination)
- ap = apathy (bees show only low or delayed reactions to stimulation [i.e. light, air blow] bees are either sitting motionless in the cage or walk but not correctly)
- m = morifold (bees cappot walk and show only very feeble movements of legs and appennae only weak response to stimulation, bees may recover but usually die)

The mortality [%] in the test item treatment was calculated from the primber of dead bees and the total number of introduced bees in the test item treatment group. The mortality of the test item treatment was corrected for corresponding control mortality, according to the formula of ABBOTT (1925), modified by SCHNEIDER-ORELLE (1947):

Corrected mortality 
$$M = \frac{t - c^{*}}{100 - c} \times 100$$

M t

Corrected mortality (%)

Mortality in the est item group %

Mortality in the control group (%)

#### Food consumption:

The consumption of feeding solution per bee/day was calculated by dividing the total daily consumption per case by the mean number of living bees at the corresponding time interval. The mean value per treatment group as well as the daily mean food consumption per bee was calculated.

The mean intake of test item per bee was calculated as follows:

Mean intake
of test item =
[μg a.i./bee]

Mean consumption of feeding solution [mg/bee]

 $X \qquad \begin{array}{c} \text{Concentration of feeding solution} \\ [\mu g \ a.i./\mu L] \end{array}$ 

Density of the sucrose solution  $[g/cm^3]$ 

# Statistical evaluations:

Fisher's Exact Test (Bonferroni-Holms corrected, one-sided,  $p \le 0.05$ ) was used to evaluate whether there are significant differences between the mortality data of the test item treatment group and the control group.

For the statistical comparison of the food consumption, non-rounded values were taken. Data of food consumption were tested for normality using Shapiro Wilks' test. It normality was observed, the t-T (one-sided,  $p \le 0.05$ ) was applied. If the data deviated from the formal distribution, statistical analysis was performed by using the Mann Whitney - Utest. Statistical calculations were made by using the statistical program SAS release Version 9.2.

# Residue analysis:

The residue analysis of the feeding solutions was performed in an independent study by Bayer , the anal Pical attached as an integral part CropScience AG,

Storage and shipment of samples:

Samples taken of the feeding solutions and the stock solution were stored deep frozen (\$\subseteq 18^{\circ}C)\$ in the

GmbM laboratory within 30 minutes. The samples were shipped on dry ice from the GmbH Maboratory directly

to the residue analysis laboratory of the sponsor (Bayer Crop Science AC)

# RESULTS AND DISCUSSION

# A. Environmental Parameters

Measurements of climatic parameters during the test are summanized actfollows:

Test temperarure: Relative humidity

except during observations Light intensity:

# B. Verification of the Application Volume

B. Verification of the Application Volume of the feeding solutions was performed in an independent study (analytical report is part of the study report, see Appendix A

# C. Biological Findings

The results of the 10 days feeding test with a control group fed with untreated sucrose solution containing 1% acetone and the test item treatment of 10000 µg a.i. /L of BYI 02960-hydroxy, first dissolved in acctone and diluted in 50% sucrose solution are presented in the table below. anuted in

# Table Mean consumption of feeding solution, mean intake of test item accumulated over all test days and cumulative mortality at the final assessment on day 10

Treatment Level <sup>1</sup>	Control	Test Item © 10000 µg a.i.
Overall mean daily consumption of aqueous sucrose feeding solution [mg/bee] <sup>2</sup>	51.4	49.6
Mean intake accumulated over test days [µg a.i./bee]	=	4,20
Cumulative mortality [%]	3.67	₹ \$ \$ £ \$ \$ £ \$ \$ £ \$ \$ £ \$ \$ £ \$ \$ £ \$ \$ £ \$ \$ £ \$ \$ £ \$ \$ \$ \$ £ \$ \$ \$ £ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$
Corrected cumulative mortality [%]		×0.66×

The control group was fed with untreated 50% (w/v) aqueous sucrose solution mixed with 1% acetorie; the test item treatment group was fed with 50% (w/v) aqueous sucrose solution containing B 402960 hydros and 1% acetone

# D. Validity Criteria

Not applicable. No guideline is available for this type of study

# E. Biological Endpoints Derived

After 10 days of continuous exposure, mortality in the test item treatment group was not significantly different compared to the control group. The cumulative control mortality accounted to 3.67%, as determined at the final assessment (day 10). In the test item treatment group at 1000@ug a.i./L, the cumulative mortality at the final assessment (day 10) accounted to 3.03% (corrected -0.66%).

Furthermore, neither subjects for behavioural abnormalities were observed throughout the entire testing period in the test item freatment group. The test item treatment level of 10000  $\mu g$  a.i./L was determined to be the NOEC (No Observed Effect Concentration, Fisher's Exact Test (Bonferroni-Holms corrected one-sided,  $p \leq 0.05$ ).

The overall mean daily consumption of the aqueous sucrose feeding solution (i.e. the average value over 10 days) in the test them treatment group was almost into the untreated control group (49.6 mg/bee in the test item treatment compared to 51.4 mg/bee in the control group).

The mean daily consumption of the aqueous sucrose feeding solution was not significantly different between the control group and the test tem treatment group throughout the entire testing period (day-by-day companion).

The accumulated nominal intake of the sest item BY102960-hydroxy via BYI 02960-hydroxy - treated aqueous sucrose feeding solution was 4.20 kg a.i. Age after 10 days of continuous exposure.

From the results presented above the following biological endpoints can be derived:

NOEC Wa.i./L

#### CONCLUSION

It can be concluded that the continuous feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item BYI 02960-hydroxy at the treatment level of 10000 µg a.i./L caused no adverse effect regarding mortality, sublethal effects and behaviour. As the overall mean daily food uptake (i.e. the average value over 10 days) in the test item treatment group was almost identical to the untreated control group and because on every single day during the 10 day continuous exposure period the mean food consumption per bee was not significantly lower in the test item treatment group

The mean values per cage over the test period were used as basis for the calculation of the overall mean daily consumption of the aqueous sucrose feeding solution per treatment over the test period.

<sup>\*</sup> Determined to be the NOEC (not significantly different compared to the control fosher's exact test; Bonferroni-Holms corrected, one-sided,  $p \le 0.05$ )

compared to the control group, it can be concluded that there was no repellent effect of the test item at the treatment level of  $10000 \, \mu g \, a.i./L$ .

The NOEC (No Observed Effect Concentration) was determined to be 10000 µg a.i./L at the end of the test period.

Report:	KIIA 8.16.1/04; (2012)	<i>a D a</i>	7
	/		
Title:	Difluoroacetic acid – Assessment of Chr	onic Effects to the Honey Bee, Apis mellifera	ı 🖇
	L., in a 10 Days Continuous Laboratory	eeding Limit Test	
Report No:	S11-01939		
Document No:	M-425105-01-1		,v (
Guidelines:	No specific guideline available 🚄		<b>1</b>
<b>Deviations:</b>	Not applicable		
GLP:	Yes (certified laboratory)		

#### **EXECUTIVE SUMMARY**

The chronic effects of the test item difluoroacetic acid Origin Batch No.: BCOO 5984-1-1; Batch Code: BCS-AA56716-01-01; TOX 09400-00 Purity 95.8% W/w) or the Koney bee, *Apic mellipera* L., in a 10 days continuous feeding limit test in the laboratory were assessed.

Over a period of 10 days, honey bees were exposed to 50% (www) agreeous vicrose deeding solution, containing nominally 10000 µg a.i./L of the test item difluoreacetic acid by continuous and ad libitum feeding. Because the test item was first dissolved in acetome and then diffuted with agreeous sucrose solution, the final test item feeding solution contained 1% acetome. The control group was exposed for the same period of time under identical exposure conditions to untreated 50% (www) aqueous sucrose feeding solution, also containing 1% acetome. Mortality, sublethal effects and behavioural observations were assessed every day throughout the 10 day sexposure period.

Samples and retain samples of all feeding solutions and the stock solution were taken for chemical analysis.

The accumulated nominal intake of the lest item diffuoroacetic acid via diffuoroacetic acid - treated aqueous sucrose feeding solution was 3.79 for a.i. see after 10 days of continuous exposure.

It can be concluded that the continuous feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item diffuoroactic act at the treatment level of 10000 µg a.i./L caused no adverse effect regarding mortality, subjected effects and behaviour. As the overall mean daily food uptake (i.e. the average value over 10 days) in the test tem treatment group was almost identical to the untreated control group and because on every single day during the 10 day continuous exposure period the mean food consumption per becomes not significantly lower in the test item treatment group compared to the control group, it can be concluded that there was no repellent effect of the test item at the treatment level of 10000 µg a.i./L

The NOEC (No Observed Effect Concentration) was determined to be 10000 µg a.i./L at the end of the test period

#### MATERIAL AND METHODS

#### A. Materials

# 1. Test material

Test item: Difluoroacetic acid (BCS-AA56716) Type of test material: Pure metabolite (technical substance)

Chemical state and description Liquid, light yellow

BCOO 5984-1-1 (Batch code:BCS-AA56716-01 Origin Batch No.: TOX 09400-00 (Test item code: 2011-001527) Sample No.:

CAS#: 381-73-7 Purity: 95.8% w/w

Stability of test compound: Expiry date: 10.2011, when stored at +10 to

50% (w// aqueous sucrose feeding solution, containing) **Treatment** 

nominally 10000 μg a GL of the test from diffuoroacetic acid as well as 1% apetone.

Control

# 2. Test organisms

Species:

Common name:

Age or developmental stage at tests

#### B. Study design and methods

1. In life dates

# 2. Experimental treatments

A study was conducted to determine the chronic effects of the test tem diffuoroacetic acid on the honey bee, Apis mellifera Loin a fordays continuous feeding limit test in the Jaboratory. The NOEC value (No Observed Effect Concentration) for mortality was determined at the end of the test period.

The hone bees were fed continuously and ad libitum with a 50% (w/v) aqueous sucrose solution, containing the test item diffuoroacetic acts at the nominal concentration level of 10000 µg a.i./L as well as 1% acetone. For the preparation of the test item treatment level, the active substance content of the test item BCS\_AA56746 (diffuoroacetic acid) was considered to be 100% and not corrected for the analysed content of 95.8% (w/w)

In the control group the boney bees received an untreated 50% (w/v) aqueous sucrose solution

During the entire test period, the bees were kepp in cages made of stainless steel (base: 8.2 cm x 4 cm; height: 6 cm). The front side of the cages were equipped with a transparent pane so that the bees could be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test mimals. The last cages are lined with filter paper.

The feeding solutions were offered to the bees in syringes fitted into the test cages.

The study was carried out with the following treatments:

- ope concentration of the test item difluoroacetic acid
- the control

The test item treatment was tested with 10 replicates (cages), each containing 10 bees (i.e. in total 100 honey bees). The control group comprised 30 replicates of 10 bees each (i.e. in total 300 honey bees).

A toxic standard was not included in this test, since a toxic reference substance had not been defined or validated for this type of study.

# 3. Observation and measurements

### Mortality:

The number of dead bees in the individual test cages was recorded every day at about the same time of day during the 10 days test period.

day during the 10 days test period.

Abnormal behavioural effects and behavioural differences were assessed according to the following categories and recorded in the raw data:

- a = affected (bees still upright and attempting to wark but showing signs of reduced coordination)
- ap = apathy (bees show only low or delayed reactions to stimulation fi.e. light, air flow) bees are either sitting motionless in the cage of walk but not correctly)
- m = moribund (bees cannot walk and show only very feeble nevembers of logs and antennae, only weak response to stimulation, bees may recover but usually die)

The mortality [%] in the test item treatment was calculated from the number of dead bees and the total number of introduced bees in the test item treatment group. The mortality of the test item treatment was corrected for corresponding control mortality, according to the formula of Apport (1925), modified by SCHNEIDER-ORELLI (1947).

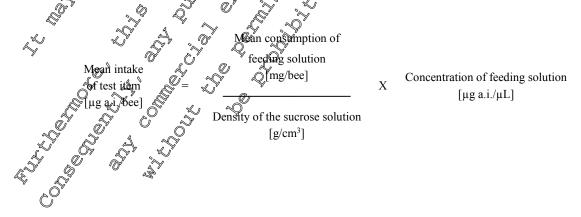
Corrected portality 
$$M = \frac{l - c}{100 c} \times \frac{c}{100 c}$$

t = Mortalis in the test item group (%)

#### Food consimption

The consumption of seeding solution per bee/day was calculated by dividing the total daily consumption per cage by the mean number of living bees at the corresponding time interval. The mean value per treatment group as well as the daily mean food consumption per bee was calculated.

The mean intake of test item per loe was calculated as follows:



#### Statistical evaluations:

Fisher's Exact Test (Bonferroni-Holms corrected, one-sided,  $p \le 0.05$ ) was used to evaluate whether there are significant differences between the mortality data of the test item treatment group and the control group.

For the statistical comparison of the food consumption, non-rounded values were taken. Data of food consumption were tested for normality using Shapiro-Wilks' test. If normality was observed, the prest (one-sided,  $p \le 0.05$ ) was applied. If the data deviated from the normal distribution, statistical analysis was performed by using the Mann Whitney – U test. Statistical calculations were made by using the statistical program SAS release Version 9.2.

#### Residue analysis:

The residue analysis of the feeding solutions was performed in an independent study by Bayor CropScience AG, Monheim, the analytical attached as an integral part of the study report.

Storage and shipment of samples:

Samples taken of the feeding solutions and the stock solution were stored deep frozen (≤ 18°C) in the

GmbH laboratory Within 30 minutes. The samples were

shipped on dry ice from the

Smb# - laboratory directly to

the residue analysis laboratory of the sponsor (Bayer CropScience AG)

# RESULTS AND DISCUSSION ©

# A. Environmental Parameters

Measurements of climatic parameters during the test are symmarized a dollows

Test temperature:

25:14-25:14C

Relative air humidity:

%4.0 – 68,8°%

Light intensity.

Constant darkness except during observations

# B. Verification of the Application Volume

The residue analysis of the feeding solutions was performed in an independent study (analytical report is part of the study report, see Appendix N3).

# C. Biological Findings

The results of the 10 days feeding test with a control group fed with untreated sucrose solution containing  $\frac{1}{10000}$  acetone and the test item freatment of 10000  $\mu g$  a.i. /L of difluoroacetic acid, first dissolved in acetone and diluted in 50% sucrose solution are presented in the table below.

# Table Mean consumption of feeding solution, mean intake of test item accumulated over all test days and cumulative mortality at the final assessment on day 10

Treatment Level <sup>1</sup>	Control	Test Item 10000 μg a.i./L	
Overall mean daily consumption of aqueous sucrose feeding solution [mg/bee] <sup>2</sup>	46.2	45.0	
Mean intake accumulated over test days [µg a.i./bee]	-	3.790	
Cumulative mortality [%]	2.33	1.00*	
Corrected cumulative mortality [%]	- 8	-1.37	Y Č

The control group was fed with untreated 50% (w/v) aqueous sucrose solution mixed with the actione; the dest item treatment group was fed with 50% (w/v) aqueous sucrose solution containing difluoroactic action 12% acetone

# D. Validity Criteria

Not applicable. No guideline is available for this type of study

# E. Biological Endpoints Derived

After 10 days of continuous exposure, nortality in the test item treatment group was not significantly different compared to the control group. The cumulative control mortality accounted to 2.33%, as determined at the final assessment (day 10). In the test item treatment level of 10000 ag a.i./L, the cumulative mortality at the final assessment (day 10) accounted to 1.00% (corrected -1.37%).

Furthermore, neither subjethal ffects nor behavioural abnormalities were observed throughout the entire testing period in the test item treatment group. The test item treatment level of 10000 μg a.i./L was determined to be the NOEC (No Observed Effect Concentration, Fisher's Exact Test (Bonferroni-Holms corrected one-sided,  $10 \le 0.050$ ).

The overall mean daily consumption of the aqueous sucrose feeding solution (i.e. the average value over 10 days) in the test trem treatment group was almost in the untreated control group (45.0 mg/bee in the test item treatment compared to 46.2 mg/bee in the control group).

The mean daily consumption of the aqueous sucrose feeding solution was not significantly different between the control group and the test tem treatment group throughout the entire testing period (day-by-day companion).

The accumulated nominal intake of the test item difluoroacetic acid via difluoroacetic acid - treated aqueous sucrose feeding solution was 3.79 kg a.i./loe after 10 days of continuous exposure.

From the results presented above the following bological endpoints can be derived:

NOEC Wa.i./L

#### CONCLUSÃON

It can be concluded that the continuous feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item difluoroacetic acid at the treatment level of 10000 µg a.i./L caused no adverse effect regarding mortality, sublethal effects and behaviour. As the overall mean daily food uptake (i.e. the average value over 10 days) in the test item treatment group was almost identical to the untreated control group and because on every single day during the 10 day continuous exposure period the mean food consumption per bee was not significantly lower in the test item treatment group

The mean values per cage over the test period were used as basis for the calculation of the overall mean daily consumption of the aqueous sucrose feeding solution per treatment over the test period.

<sup>\*</sup> Determined to be the NOEC (not significantly different compared to the control fosher's exact test; Bonferroni-Holms corrected, one-sided,  $p \le 0.05$ )

compared to the control group, it can be concluded that there was no repellent effect of the test item at the treatment level of  $10000 \, \mu g \, a.i./L$ .

The NOEC (No Observed Effect Concentration) was determined to be 10000 µg a.i./L at the end of the test period.

Report:	KIIA 8.16.1/05; (2012)	3 5	
Title:	6-chloronicotinic acid – Assessment of C	hronic Effects to the Honey Bee, <i>pis m</i>	eflifera 🗸
	L., in a 10 Days Continuous Laboratory	eeding Limit Test	
Report No:	S11-01957		
Document No:	M-425155-01-2	Q Q	
Guidelines:	No specific guideline available		
<b>Deviations:</b>	Not applicable		_@"
GLP:	Yes (certified laboratory)		

#### **EXECUTIVE SUMMARY**

The chronic effects of the test item 6-chlosonicotrinic acid (Origin Batch No.: M12653; Batch code AE F161089 00 1B99 0001; Purity 98.8% www on the honey bee, \*\*pis melliferro\*\*., in & 10 days continuous feeding limit test in the laboratory were assessed.

Over a period of 10 days, honey bees were exposed to 50% ( $\sqrt[6]{v}$ ) agreeous incrose deeding solution, containing nominally  $10000~\mu g$  a.i./L of the test item 6-chloronic dark acid, by continuous and ad libitum feeding. Because the test item was first dissolved in acetone and then diluted with aqueous sucrose solution, the final test item feeding solution contained 1% acetone. The control group was exposed for the same period of time under identical exposine conditions to unificated 50% (w/v) aqueous sucrose feedling solution, also containing 1% acctone. Mortality, sublethal effects and behavioural observations were assessed every day throughout the 1% days exposure period.

Samples and return samples of all feeding solutions and the stock solution were taken for chemical analysis.

The accumulated nominal intake of the sest item 6-chloronicatinic acid via 6-chloronicotinic acid treated aqueous sucross soluiton was 4.18 gg a.i./bee after 10 days of continuous exposure.

It can be concluded that the continuous feeding of honey bees in the laboratory over a period of 10 consecutive days with the test atem 6 chloroficotinic acid at the treatment level of 10000 µg a.i./L caused no adverse effect regarding mortality, subjethal effects and behaviour. As the overall mean daily food uptake (i.e. the average value over 10 days) in the test item treatment group was comparable to the untreated control group and because on every single day during the 10 day continuous exposure period the mean food consumption per because not significantly lower in the test item treatment group compared to the control group, it can be concluded that there was no repellent effect of the test item at the treatment level of 10000 µg a.i./

The NOEC (No Observed Frect Concentration) was determined to be 10000 µg a.i./L at the end of the test period

#### MATERIAL AND METHODS

#### A. Materials

# 1. Test material

Test item: 6-chloronicotinic acid (AE F161089)

Type of test material: Pure metabolite Chemical state and description Beige powder

M12653 (Batch code: AE F161089 @ 1B99 0001) Origin Batch No.: Not available (Test item code: 201 001593) Sample description:

CAS#: 5326-23-8 Purity: 98.8% w/w

Stability of test compound: Expiry date: 207.2012, when stored at +5

50% (w/ Paqueous sucrose feeding solution, containing **Treatment** 

nominally 10000 μg a ML of the test icom 6-chloronicotinic acid as well as 1% acctore

**Control** 

#### 2. Test organisms

Species:

Common name:

Age or developmental stage at test

#### B. Study design and method

1. In life dates

#### 2. Experimental treatments

A study was conducted to determine the chronic effects of the test tem 6-chloronicotinic acid on the honey bee, Apis mellifera L., In a 10 days continuous feeling limit test in the laboratory. The NOEC value (No Observed Effect Concentration) for mortality was determined at the end of the test period.

The honey bees were red continuously and ad libitum with a 50% (w/v) aqueous sucrose solution, containing the test item 6-chloronic ottinic acid at the nominal concentration level of  $10000~\mu g$  a.i./L as well as 1% acetone. For the preparation of the test item treatment level, the active substance content of the test item AFF161689 (6-Chloron cotinic acid) was considered to be 100% and not corrected for the analysed content of 98.8% (W/w)

In the control group the coney bees received in untroated 50% (w/v) aqueous sucrose solution containing 1% acetone, ad libitum.

The feeding solutions were offered ad libitum of each cage of 10 bees in plastic syringes ( Germany). The tip of each syringe was removed so that the bees had access to the reding solution. Every morning the syringes of all test cages (i.e. test item and control) were replaced by new symples. Filled with freshly prepared feeding solution. The weight of the syringes was determined before and after feeding on the next day in order to determine the mean food consumption of the bees per test cage.

During the entire test period, the bees were kept in cages made of stainless steel (base: 8.2 cm x 4 cm; height: 6 cm). The front side of the cages were equipped with a transparent pane so that the bees could

be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test animals. The test cages are lined with filter paper.

The feeding solutions were offered to the bees in syringes fitted into the test cages.

The study was carried out with the following treatments:

- one concentration of the test item 6-chloronicotinic acid
- the control

The test item treatment was tested with 10 replicates (cages), each containing 10 bees (i.e. in total 1900 honey bees). The control group comprised 30 replicates of 10 bees each (i.e. in total 2000 honey bees).

A toxic standard was not included in this test, since a wxic reference substance had not been defined a validated for this type of study.

### 3. Observation and measurements

# **Mortality**

The number of dead bees in the individual test cases was recorded every day of about the same time of day during the 10 days test period.

Abnormal behavioural effects and behavioural differences were assessed according to the following categories and recorded in the raw data:

- a = affected (bees still upright and attempting to walk but showing signs of educed coordination)
- ap = apathy (bees show only low or delayed reactions to stimulation [i.e. fight, air blow] bees are either sitting motionless in the cage or walk but not correctly)
- m = morigand (bees cannot walk and show only very feeble movements of legs and antennae only weak response to stimulation. Bees may recover but usually die)

The mortality of in the test item treatment was calculated from the number of dead bees and the total number of introduced bees in the test item treatment group. The mortality of the test item treatment was corrected for corresponding control mortality, according to the formula of ABBOTT (1925), modified by SCHNEIDER-ORELLI (\$447):

Corrected mortality 
$$M = 100 \div 100$$

= Corrected mortality  $M = 100 \div 100$ 

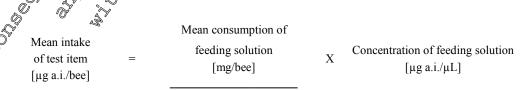
t = Mortality in the test tem group (%) c = Mortality in the control group (%)

## Food consumption

M

The consumption of feeding solution for bee-day was calculated by dividing the total daily consumption per cage by the mean number of living bees at the corresponding time interval. The mean value per treatment group as well as the Gaily mean food consumption per bee was calculated.

The mean mtake of test item per bee was calculated as follows:



Density of the sucrose solution [g/cm<sup>3</sup>]

# **Statistical evaluations:**

Fisher's Exact Test (Bonferroni-Holms corrected, one-sided,  $p \le 0.05$ ) was used to evaluate what there are significant differences between the mortality data of the test item treatment group and t control group.

For the statistical comparison of the food consumption, non-rounded values were taken. Data of food consumption were tested for normality using Shapiro-Wilks' test. If normality was observed, the tore (one-sided,  $p \le 0.05$ ) was applied. If the data deviated from the normal distribution, statistical analys was performed by using the Mann Whitney – U test Statistical calculations were made by using the statistical program SAS release Version 9.2.

# Residue analysis:

The residue analysis of the feeding solutions was performed in an independent study by Baye CropScience AG, Monheim, the analytical attached as an integral part of the study report

# Storage and shipment of samples:

Samples taken of the feeding solutions and the stock solution were stored deep frozen ( $\leq -18^{\circ}$ C) in the

GmbH laboratory within 30 minutes. The camples were shipped on dry ice from the ZmbH\_Plaboratory directly to the residue analysis laboratory of the sponsor (Bayer CopScience Ac)

# RESULTS AND DISCUSSIO

# A. Environmental Karameters

Measurements of Climatic parameters during the test are

Relative air bumidity: 25.1 – 25.4°© 64.44 - 68.8

Constant darkness except during observations

# B. Verification of the Application Volume

The residue analysis of the feeding solutions was performed in an independent study (analytical report is part of the stody report, see Appendix AS.

# C. Biological Findings

The results of the 10 days feeding test with control group fed with untreated sucrose solution containing 1% acetone and the test frem treatment of 10000 µg a.i. /L of 6-chloronicotinic acid first containing 170 accurate and diluted in 50% sucrose solution are presented in the table below.

#### **Table** Mean consumption of feeding solution, mean intake of test item accumulated over all test days and cumulative mortality at the final assessment on day 10

Treatment Level <sup>1</sup>	Control	Test Item 10000 µg a.i./L
Overall mean daily consumption of aqueous sucrose feeding solution [mg/bee] <sup>2</sup>	46.2	49.7
Mean intake accumulated over test days [µg a.i./bee]	=	04.18
Cumulative mortality [%]	2.33	3.00*
Corrected cumulative mortality [%]	- <sub>&amp;</sub>	0.69

The control group was fed with untreated 50% (w/v) aqueous sucress feeding solution mixed with 1% acetons the test. item treatment group was fed with 50% (w/v) aqueous sucrose feeding solution containing 6-chloronicotinic acid and 1% acetone

# D. Validity Criteria

Not applicable. No guideline is available for this

# E. Biological Endpoints Derived

After 10 days of continuous exposure, mortality in the test item freatment group was not significantly different compared to the control group. The cumulative control mentality accounted to \$33 %, as determined at the final assessment (day 10) on the test item treatment group at \$0000, µg a.i./L, the cumulative mortality at the final assessment (day 10) accounted to 3.00% (corrected 0.69%).

Except for one moribund bee at the assessment after 4 days (44) and one affected bee at the assessment after 5 days (d5), neither sublethal offects for behavioural abnormalities were observed throughout the entire testing period in the dest item treatment group. The testinem treatment evel of 10000 µg a.i./L was determined to be the NOEC (No Observed Effect Concentration, Fisher's Exact Test (Bonferroni-Holms corrected, one sided  $p \le 0.05$ ).

The overall mean daily consumption of the aqueous sucrose feeding solution (i.e. the average value over 10 days) in the test item the atment group was comparable to the untreated control group (49.7) mg/bee in the test item treatment compare to 46.2 mg/bee in the control group).

The mean daily consumption of the appeous sperose feeding solution was not significantly different between the control group and the test item Peatment group throughout the entire testing period (dayby-day comparison).

The accuraliated nominal intake of the lest item 6-chloronicotinic acid via 6-chloronicotinic acid treated aqueous sucrose solution was 4.18 pg a.i. Lee after 10 days of continuous exposure.

From the results presented above the following Diological endpoints can be derived:

**NOEC** 

It can be conclosed that the continuous feeding of honey bees in the laboratory over a period of 10 consecutive days with the sest item 6-chloronicotinic acid at the treatment level of 10000 µg a.i./L caused no diverse effect regarding mortality, sublethal effects and behaviour. As the overall mean daily food uptake (i.e. the average value over 10 days) in the test item treatment group was comparable to the untreated control group and because on every single day during the 10 day continuous exposure period the mean food consumption per bee was not significantly lower in the test item treatment group

The mean values per cage over the test period were used as basis for the calculation of the overall mean daily consumption of the aqueous sucrose feeding solution per treatment over the text period

Fisher's Exact Test Bonferroni-Halms Determined to be the NOEC (not significantly different compared to the corrected, one-sided,  $p \le 0.05$ )

compared to the control group, it can be concluded that there was no repellent effect of the test item at the treatment level of  $10000 \, \mu g \, a.i./L$ .

The NOEC (No Observed Effect Concentration) was determined to be 10000 µg a.i./L at the end of the test period.

			U/	
Report:	KIIA 8.16.1/06; (201		, S	
Title:	6-chloropicolyl alcohol – Assessmen mellifera L., in a 10 Days Continuou	t of Chronic Effects to the S Laboratory Feeding Li	ne Honey Bee Api mit Test	
Report No:	S11-01958	, * S	. O S	
Document No:	M-425159-01-2	4	_0~~~	O
<b>Guidelines:</b>	No specific guideline available	Q &°		
<b>Deviations:</b>	Not applicable	~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~		) <u>"</u>
GLP:	Yes (certified laboratory)			

#### **EXECUTIVE SUMMARY**

The chronic effects of the test item 6-chloropically alcohol (Ofigin Barch Nov. M06773; Barch code: AE F157983-PU-01; Purity 99.4% w/s on the honey bee opis methigera. in a 10 days continuous feeding limit test in the laboratory were assessed.

Over a period of 10 days, honey bees were exposed to 50% ( $\sqrt{v}$ ) agreeous across feeding solution, containing nominally 10000  $\mu g$  a.i./L of the est item 6-chloropically alcohol by continuous and ad libitum feeding. Because the test item was birst dissolved in acetone and then diluted with aqueous sucrose solution, the final test item feeding solution contained 1% acetone. The control group was exposed for the same period of time under identical exposure conditions to untreated 50% ( $\sqrt{v}$ ) aqueous sucrose feeding solution, also containing 1% acetone. Mortality, sublethal effects and behavioural observations were assessed every day throughout the 10 days exposure period.

Samples and return samples of all feeding solutions and the stock solution were taken for chemical analysis.

The accurant ated nominal intake of the test item 6-chloropicolyl alcohol via 6-chloropicolyl alcohol - treated aqueous sucrose solution was 4.13 mg a.i. bee after 10 days of continuous exposure.

It can be concluded that the continuous feeding of loney bees in the laboratory over a period of 10 consecutive days with the test item  $\mathscr G$ -chloropicolol alcohol at the treatment level of 10000  $\mu g$  a.i./L caused no adverse effect regarding mortality, sublethal effects and behaviour. As the overall mean daily food uptake (i.e. the average value over 10 days) in the test item treatment group was comparable to the untreated control group and because only on one single day during the 10 day continuous exposure period the mean food consumption perbee was significantly lower in the test item treatment group compared to the control group it can be concluded that there was no repellent effect of the test item at the treatment level of 10000  $\mu g$  a.j./L.

The NOEC (No observed Effect Concentration) was determined to be 10000 µg a.i./L at the end of the test period.

#### MATERIAL AND METHODS

#### A. Materials

1. Test material

Test item: 6-chloropicolyl alcohol (AE F157983)

Type of test material: Pure metabolite Chemical state and description Beige crystals

M06773 (Batch code: AE F157983-PU=01) Origin Batch No.: Not available (Test item code: 2011 001616) Sample description:

CAS#: 21543-49-7 Purity: 99.4% w/w

Stability of test compound: Expiry: 12.11.2017, when stored at  $+5 \pm 5$ 

50% (w/v) flueous sucrose feeding solution containing & **Treatment** 

nominally 10000 µg a.i. Wof the rest item 6-chtoropically alcohol as well as 1% acctone.

sucrose seeding solution containing 1% **Control** 

### 2. Test organisms

Species:

Common name:

Age or developmental stage at test start

#### B. Study design and method

1. In life dates

#### 2. Experimental treatments

A study was conducted to determine the chronic effects of the test item 6-chloropicolyl alcohol on the honey bee, Apis mellifera L., In a 10 days continuous feeling limit test in the laboratory. The NOEC value (No Observed Effect Concentration) for mortality was determined at the end of the test period.

The honey bees were red continuously and ad libitum with a 50% (w/v) aqueous sucrose solution, containing the test item 6-chloropicolyl abcohol at the nominal concentration level of 10000 µg a.i./L as well as 1% acetone. For the preparation of the test item treatment level, the active substance content of the test item AFF157883 (6-Chloropycolyl arcohol) was considered to be 100 % and not corrected for the analysed content of 99.0% (wow).

In the control group the boney bees received in untroated 50% (w/v) aqueous sucrose solution containing 1% acetone, ad libitum.

The feeding solutions were offered ad libitum of each cage of 10 bees in plastic syringes ( Cermany). The tip of each syringe was removed so that the bees had access to the ceding solution. Every morning the syringes of all test cages (i.e. test item and control) were replaced by new symples. Filled with freshly prepared feeding solution. The weight of the syringes was determined before and after feeding on the next day in order to determine the mean food consumption of the bees per test cage.

During the entire test period, the bees were kept in cages made of stainless steel (base: 8.2 cm x 4 cm; height: 6 cm). The front side of the cages were equipped with a transparent pane so that the bees could

be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test animals. The test cages are lined with filter paper.

The feeding solutions were offered to the bees in syringes fitted into the test cages.

The study was carried out with the following treatments:

- one concentration of the test item 6-chloropicolyl alcohol
- the control

The test item treatment was tested with 10 replicates (cages) each containing 10 bees (se. in total 100 honey bees). The control group comprised 30 replicates of 10 bees each (i.e. in total 200 honey bees).

A toxic standard was not included in this test, since a wice reference substance had not been defined or validated for this type of study.

### 3. Observation and measurements

# **Mortality**

The number of dead bees in the individual test cases was recorded every day of about the same time of day during the 10 days test period.

Abnormal behavioural effects and behavioural differences were assessed according to the following categories and recorded in the raw data:

- a = affected (bees still upright and attempting to walk but showing signs of educed coordination)
- ap = apathy (bees show only low or delayed reactions to stimulation [i.e. light, air blow] bees are either sitting motionless in the cage or walk but not correctly)
- m = morigand (bees cannot walk and show only very feeble movements of legs and antennae only weak response to stimulation. Bees may recover but usually die)

The mortality of in the test item treatment was calculated from the number of dead bees and the total number of introduced bees in the test item treatment group. The mortality of the test item treatment was corrected for corresponding control mortality, according to the formula of ABBOTT (1925), modified by SCHNEIDER-ORELLI (\$447):

Corrected mortality 
$$M = \frac{t^2 - c}{100 - c}$$
 100

= Corrected mortality  $M = \frac{t^2 - c}{100 - c}$  100

Mortality in the test tem group (%)

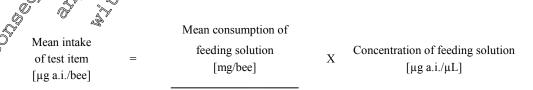
c = Mortality in the control group (%)

# Food/consumption

M

The consumption of feeding solution for bee-day was calculated by dividing the total daily consumption per cage by the mean number of living bees at the corresponding time interval. The mean value per treatment group as well as the Gaily mean food consumption per bee was calculated.

The mean mtake of test item per bee was calculated as follows:



Density of the sucrose solution [g/cm³]

# **Statistical evaluations:**

Fisher's Exact Test (Bonferroni-Holms corrected, one-sided,  $p \le 0.05$ ) was used to evaluate whether there are significant differences between the mortality data of the test item treatment group and the control group.

For the statistical comparison of the food consumption, non-rounded values were taken. Data of food consumption were tested for normality using Shapiro-Wilks' test. If normality was observed, the prest (one-sided,  $p \le 0.05$ ) was applied. If the data deviated from the normal distribution, statistical analysis was performed by using the Mann Whitney – U test Statistical calculations were made by using the statistical program SAS release Version 9.2.

# Residue analysis:

The residue analysis of the feeding solutions was performed in an independent study by Bayer CropScience AG.

# Storage and shipment of samples:

Samples taken of the feeding solutions and the stock solution were stored deep frozen  $(\leq -18^{\circ}\text{C})$  in the

GmbH laboratory within 30 minutes. The samples were

shipped on dry ice from the

GnabH - laboratory directly to

the residue analysis laboratory of the sponsor (Rayer CopScience AC)

# RESULTS AND DISCUSSION

# A. Environmental Parameters

Measurements of climatic parameters during the test are simmadized as follows:

Test temperature:

25 25.4°C

Relative humidity:

68.89

Light intensity:

Constant darkness except during observations

# B. Verification of the Application Volume

The residue analysis of the feeding solutions was performed in an independent study (analytical report is part of the study report, see Appendix (3).

## C. Biological Findings

The results of the 10 days feeding test with a control group fed with untreated sucrose solution containing 1% acetone and the test item treatment of 10000  $\mu g$  a.i. /L of 6-chloropicolyl alcohol, first dissolved in acetone and diluted in 50% sucrose solution are presented in the table below.

#### **Table** Mean consumption of feeding solution, mean intake of test item accumulated over all test days and cumulative mortality at the final assessment on day 10

Treatment Level <sup>1</sup>	Control	Test Item
Overall mean daily consumption of aqueous sucrose feeding solution [mg/bee] <sup>2</sup>	46.2	4071
Mean intake accumulated over test days [µg a.i./bee]	-	04.13
Cumulative mortality [%]	2.33	5.00*
Corrected cumulative mortality [%]	-	2.73

The control group was fed with untreated 50% (w/v) aqueous sucrose feeding solution mix with 4% acetone; the test item treatment group was fed with 50% (w/v) aqueous sucrose feeding solution containing 6 chloropicolyl alcohol and 1% acetone

- The mean values per cage over the test period were used as basis for the calculation of the overall mean dails consumption of the aqueous sucrose feeding solution per treatment over the tes Preriod
- Determined to be the NOEC (not significantly different compared to the control, fosher's exact Test; Bonferroni-Holms corrected, one-sided,  $p \le 0.05$ )

# D. Validity Criteria

Not applicable. No guideline is available for this type of

# E. Biological Endpoints Derived

After 10 days of continuous exposure, mortality in the test it in treatment group was not significantly different compared to the control group. The cumulative control portality accounted to 2.33%, as determined at the final assessment (ay 10). In the test item treatment group at 1000@ug a.i./L, the cumulative mortality at the final assessment (day 10) accounted to 5.00 % corrected 2.73%).

Furthermore, neither subjethal frects nor behavioural abnormalities were observed throughout the entire testing period in the test item treatment level of 10000 µg a.i./L was determined to be the NOEC(No Observed Effect Concentration, Fisher's Exact Test (Bonferroni-Holms corrected one-stided, post 0.050).

The overall mean daily consumption of the aqueous sucrose feeding solution (i.e. the average value over 10 days) in the test frem the atment group was comparable to the untreated control group (49.1 mg/bee in the test item treatment compared to 46.2 mg/bee in the control group).

The mean daily consumption of the aqueous sucrose feeding solution was statistically significantly reduced in the test item reatment group (46 mg/bee) compared to the control group (55 mg/bee) only on day 1 (t- test pooled; one gided;  $p \le 0.05$ ). On all other test days, the mean daily consumption of the aqueous sucrose feeding solution was althost identical or even higher in the test item treatment group compared to the control group (day by-day comparts on).

The accumulated nominal intake of the test item o-chloropicolyl alcohol via 6-chloropicolyl alcohol sucrose solution was 4013 µg/h/1./bee after 10 days of continuous exposure. treated aqueous

presented above the following biological endpoints can be derived:

10000 µg a.i./L

It can be concluded that the continuous feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item 6-chloropicolyl alcohol at the treatment level of 10000 µg a.i./L caused no adverse effect regarding mortality, sublethal effects and behaviour. As the overall mean daily

food uptake (i.e. the average value over 10 days) in the test item treatment group was comparable to the untreated control group and because only on one single day during the 10 day continuous exposure period the mean food consumption per bee was significantly lower in the test item treatment group compared to the control group, it can be concluded that there was no repellent effect of the test item at the treatment level of 10000 µg a.i./L.

the treatment level of 10000 µg a.1./L.

The NOEC (No Observed Effect Concentration) was determined to be 10000 µg a.i./L at the end of the test period.

Report:	KIIA 8.16.1/07; A., M., D.; (2010)
Title:	BYI 02960 tech.: Effects of exposure to spiked diet on honey beet Apis mellifera
	carnica) larvae in an in vitro laboratory testing design.
Report No:	E 318 3897-9
Document No:	M-406645-01-2
Guidelines:	No validated guideline available Study design according to the recommendations of
	the INRA (Institut National della Recherche Agronomique) method for testing @
	pesticide toxicity to honey bee brood in laboratory conditions (January, 2008) and
	the recommendations of the honey bee larvae laborators ring-test group, organized
	by ICPBR (Aupmel et 41, 2009) S S S S
Deviations:	Not applicable V V V V V V V V V V V V V V V V V V V
GLP:	Yes (certified laboratory) Q Q Q Q Q Q
	The rearing of bee threae in the beginives was not part of GLP. The preparation of
	saturated solutions of KSO4 and NaCl and the preparation of solutions for the
	disinfection of grafting cells as well as for the wetting of dental rolls of ere not part of
	GLP. The procedure of the disinfection of grafting colls and the preparation of the
	rearing plates, respectively test plates were not part of the GLP.

# EXECUTIVE SUMMARA

The purpose of the biological part of this study was to assess the effects of BYI 02960 tech. (TOX 08508-00, Specification No.: 102000022310, Batch code BYI 02960-01-03; content of a.i. (analysed): 96.2% w/w) on honey bee lat vae, Apris mellifera carnical after artificial feeding of spiked diet in art of vitro laboratory testing design. The purpose of the analytical part of this study was to quantify the concentration of BYI 02960 in spiked exposure diets, which were used to feed the larvae in the biological part of this study.

At day +1 (day 6) was the anticipated day of darval hatching), first instar bee larvae (*Apis mellifera carnica*) were transferred from their bee lave into an artificial *in vitro* testing system. The bee larvae were fed with standardised amounts of intreated artificial diet at day +1 and day +3. On day +4, +5 and +6, the bee larvae in the test item treatment groups were fed with standardized amounts of test item spiked artificial exposure diet. On day +4, the bee larvae in the reference item treatment group were fed with standardised amounts of reference item spiked artificial exposure diet. Concurrently, the bee larvae in the control group (on day +4, +5 and +6) and in the reference group (on day +5 and +6) received untreated artificial exposure diet, respectively. In the test item treatment groups, BYI 02960 (tech.) was incorporated into the artificial exposure diet at the nominal test concentrations of 150, 600, 2500 and 10000 per a.i./kg diet, respectively. The actual concentration of BYI 02960 in the test item spiked exposure diet was determined according to analytical method 01206 by using High Performance Liquid Chromatography, coupled with tandem mass spectrometry.

During the development of the honeybee larvae, the larvae were incubated at about +35°C. From day +1 to +8, the relative humidity inside the incubator was on average about  $95 \pm 5\%$  and from day +8 to +22 the mean relative humidity was about  $80 \pm 5\%$ .

Mortality was determined on day +5, +6, +7, +8, +11, +13, +15 and +22. Dead test animals were discarded for sanitary reasons.

Five independent test runs were performed, from which 3 fulfilled both the INRA and the selvalidity criteria.

Overall, it can be concluded that the No Observed Effect Concentration (NOEC) as defermined in this in vitro honeybee larvae study is ≥ 10000 µg BYI 02960 a.i./kg diet.

MATERIAL AND METHODS

A. Materials

1. Test material

Test item:

Type of test material:
Chemical state and description:
Origin Batch No.:
Specification No.:
Specification No.:
Specification No.:
Sample description:
CAS name:

CAS#:

ON CAS 10000 µg BYI 02960 va.i./kg diet.

BYI 02960 va.i./kg diet.

Substance, technical
Beige powder
2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-000239

2009-

CAS#: 931659-49-8 96.2% (agailytical) Purity:

4-[(6-chloropyridin Sylmetbyl)(22-difluoroethyl) mino] furan-2(5H)-IUPAC name:

Expiry date: 16.01.2011 when stored at  $+25 \pm 5^{\circ}$ C Stability of test com

Artificial exposure diet containing the test item BYI 02960 at the **Treatment** nominal test concentrations of 150, 600, 2500 and 10000 µg a.i./kg diet

Dimethoate technical Reference Item

Purity: Nominal text concentration 3.0 kg a.i./larva

Artificial Tet consisting of 50% noval jelly and 50% of an aqueous Basic larval diet solution containing yeast extract, glucose and fructose in different (= negative control

tions (dependent on the day of administration)

#### 2. Test organisms

its/mellifera carnica Species:

Common name:

Age of developmental rage at less Source: First in far larvae, 1 day after hatching

Locobeekeeper

June 14 to August 27, 2010

Principle of the testing procedure: At day +1°, first instar bee larvae (*Apis mellifera carnica*) were transferred from their bee hive into an artificial *in vitro* testing system. The bee larvae were fed with standardised amounts of untreated artificial diet at day +1 and day +3. On day +4, +5 and +6, the bee larvae in the test item treatment groups were fed with standardised amounts of test item spiked artificial exposure diet. On day +4, the bee larvae in the reference item treatment group were fed with standardised amounts of reference item spiked artificial exposure diet. Concurrently, the bee larvae in the control group (on day +4, +5 and +6) and in the reference group (on day +5 and +6) received untreated artificial exposure diet, respectively. In the test item treatment groups, BYI 02960 (tech.) was incorporated into the artificial exposure diet at the nominal test concentrations of 150 600, 200 and 10000 μg a.i./kg diet. The actual concentration of BYI 2960 in the test item spiked exposure diet was determined according to analytical method 01206 by using High Performance Liquid Chromatography, coupled with tandem mass spectrometry.

During the development of the honeybee larvae the lawae were inchated at about 35%

The feeding of the larvae took place once a day (except of day ±2) according to the following schedule

Table: Feeding regime of the honey bee farvae

Treatment	Day	+1 0	§×+2 <sub>∞</sub>	F3 \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\	(PA		46
group	Diet Type	A,O	n.ą.	B 🔊 🔏	c o	<u>, , , , , , , , , , , , , , , , , , , </u>	<b>V</b>
Control	Volume of	20 4	nĢă.	20	30 %	40 (universated)	<sup>"</sup> 50
Control	diet/larvae [μL] ≪	(untreated)	119a.	(Ontreated)	(prereated)	(untreated)	(untreated)
Test item	Volume of	200/2	× ~ ~	,20	430 ×	Ø\$0	50
1 est item	diet/larvae [🏚]	(untreated)	n.a	(untreated)	(negreta)	√(treated)	(treated)
Reference	Volume of *>	<u>√</u> 20	J.a.	20 ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° °	30	40	50
Reference	diet/larvæ[µL] 《	∀(untr <b>©a</b> ted)	Ora.	Juntreated)	(treated)	(univereated)	(untreated)

n.a.: not applicable

# 3. Observation and measurements

Time table of mortality assessments:

Larva: Before start of exposure trie. from day +1 until day +4), dead larvae were systematically removed for sanitary reasons. Exposure of the larvae to the treated diet started on day +4; during exposure (i.e. from day +4 thatil day +6) and after exposure, mortality was determined on day +5, +6, +7 and +8. Dead larvae were recorded and thereafter discarded.

<u>Pupa:</u> Not emerged bees were counted on day +22. During the pupal stage, obviously dead pupae were recorded and thereafter discanded on several dates for sanitary reasons.

Adult: Energed adult bees were counted on any +22

# RESULTS AND DISCUSSION

# A. Environmental Parameters

<sup>9</sup> Day 0 was the anticurated day of large hatching

Remark this starty is designed to address chronic exposure of bee larvae to the test item. However, since a reliable design for a full thronic exposure (i.e. feeding with standardised amounts of treated artificial diet from day +1 to +6) has not yet been developed nor ing-tested or valuated, and no appropriate validity criteria are so far defined for control mortality during day +1 to +3 (in this phase, very high control mortalities are regularly observed), the exposure of the bee larvae to the test item will take place in this study from day +4 onwards until day +6, where more stable conditions in terms of control mortality are to be expected.

From day +1 to day +8, the larvae were incubated in a hermetic container at about +35°C, containing a dish filled with a saturated solution of  $K_2SO_4$  in order to keep a water saturated atmosphere of on average 95 ± 5% relative humidity. For the control group a separate incubator with the same test conditions as described above was used from day +4 to day +8, to avoid any potential contamination of the control larvae. From day +8 until day +22, the larvae were transferred into a permetic container at about +35°C, containing a dish with a saturated solution of NaCl in order to keep a relative humidity of on average  $80 \pm 5\%$ .

The desired test conditions were recorded with suitable and alibrated instruments and the obtained measurements are documented in the raw data.

The experimental unit was the 48-well culture plate made of polystorene. For the test, the following test groups were used:

- Control group: 1 plate
- Test item treatment group: Four test concentrations, 1 plate per lest concentration, respectively
- Reference group: Dimethoate, 1 plates

The nominal test concentrations and the calculated, nominal feeding rates of the test groups used in this study are presented in following table:

Table: Feeding rates of honey bee large during the exposure period

	Treatment on:		yaay 12	<b>day +6</b>
Treatment	- 9 1 4	30 mg) 30 mg/30 mg/	40 µ()	50 μL (≈ 55 mg)
groups	Freatment level	1 41% .2 *	Feeding rate	
Control	n.a.	ina. J	n, a	n.a.
8			0.0066	0.00825
Test item		<b>₹</b> 0.019 <b>8</b> (	<b>©</b> .0264	0.033
	2500	0.08 <b>2</b> 5	0.11	0.1375
	© 100 <b>00</b>	<b>20</b> 33	0.44	0.55
Reference	90900	3.0	n.a.	n.a.

n.a.: not applicable

#### B. Verification of the Application Volume

For the analytical determination of the content of BYI \$2960 in the test item spiked exposure diets, samples of the remaining spiked diets were intrindiately deep-frozen, after feeding of the honeybee larvae on day +4, +5 and +6 was completed. The samples were labelled with the study number and all necessary additional information to assure unmistakable identification. The actual concentration of BYI 02960 in the test item spiked exposure diet was determined according to analytical method 01206, by using High Performance Liquid Chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS) and electrospray onization (ESI).

The analytical defermination of the BYI 02960 concentration in the spiked exposure diets of the test item treatment group revealed for all five test runs [test runs 2, 4 and 5] the following results:

150 μg a.i./kg diet - treatment level:

On average 95 - 118% [test runs 2, 4 and 5; 95 - 109%] of nominal

On average 100 - 140% [test runs 2, 4 and 5; 100 - 110%] of nominal

On average 87 - 106% [test runs 2, 4 and 5; 87 - 104%] of nominal

On average 88 - 108% [test runs 2, 4 and 5; 88 - 108%] of nominal



### C. Biological Findings

Table: Control and test item performance and associated statistical evaluation:

Test object	Honeybee larvae (Apis mellifera carnica)						
	Control Test item			Reference item			
	(untreated   (BYI 02960 - spiked 🄝		(dimethoate -				
	exposure	exposu	ıre diet)	)		spiked exposure	
	diet)		1	4		diet)	
Test concentration (μg a.i./kg diet)		150	600	2500	10000	3.0 (pg a.i./pr	va)
Test run No. 1 <sup>a</sup>		Ĉ'n		Š			
Mortality until day +22 [%]	31.3 a	<b>3</b> 9.6	29.2	<b>%</b> 1.7	45.8	© \$9.6 <sub>k</sub>	
Abbott-corrected mortality until day +22 [%]	0.0	, 12.1	-3.0€	15.2	21.2	84.8	Q (
Test run No. 2	<u> </u>		Ő,	, 0			"W
Mortality until day +22 [%]	16.70°	31.0	38.1	<b>2</b> 4.4	₽ <b>6</b> .7	100	
Abbott-corrected mortality until day +22 [%]	0.0	.17.1 .4	$\sqrt{2}5.7$	¥ 5.7 €	$\sim 0.0$	\ \\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	Ŋ
Statistical comparison to the control <sup>b</sup>	¥ 0,	$n.s_{\epsilon}$	n.sc	n.s	n.s	~ <sup>~</sup>	
NOEC <sup>b</sup>		.00 <b>00</b> μ	g a Økg	die	6		a . °
LOEC b	A . O > 1	-Q000 μ	g a.v./kg	diet	S		Ű
Test run No. 3 a		y	) L	7 %			7
Mortality until day +22 [%]	_ & 32.4 ° €	40∕. <b>≶</b> ″	35.)	40%	4806	\$ 91. <b>9</b>	
Abbott-corrected mortality until day +22 0 3	<b>9.0</b>	12.0	<b>40</b> .0	12.0	<b>24</b> .0	<b>88</b> .0	
Test run No. 4	~ ~	~ ~	D" (		Ŭ ź		
Mortality until day +22 [%]	₩8.8 £	16 <i>7</i>	10.4	16%	10.	95.8	
Abbott-corrected mortality until dwy+22 [%]	0.0	-25,6	-10.3	-2.6	-10.3	94.9	
Statistical comparison to the control b &	S{-	n.s.	n.s.	-2.6 ≫n.s.	Øn.s.		
NOEC b			a.i./kg	diet 🦽	) V		
LOEC "		0000942	g a.j./kg	diet∜			
Test run No. 5		~~ ~~		Q,			
Mortality until day +220%	286	<b>3</b> 1.0	26.2	$O_{31.0}$	<sup>™</sup> 40.5	100	
Abbott-corrected morphity up day 2 [%]	~0.0 °	3.3%	-3.2	3.3@	16.7	100	
Statistical comparison to the control (	~ <u> </u>	n:s.	p.S.	л.ў.	n.s.		
NOEC b S	<i>"0"</i> ≥1	000 με	gæl./kg	diet			
LOEC b	/ _ `~ > <u>†</u>	9000 με	a.i./kg	diet	1.0		

<sup>&</sup>lt;sup>a</sup>Although control performance met the validary criteria as stated in the INRA - method for testing pesticide toxicity to coneybee brood in laboratory conditions (January 2008), the self-set validity criterion for control performance at the end of the test (i.e.  $\leq 30\%$ ) was not met; no distinct differences in larval mortality can be observed at concentrations of the to antincluding 10000  $\mu$ g BVI 02960 a.i./kg diet (as the self-set validity criterion was not met, no detailed statistical evaluation is presented however, when subjecting the data to statistical analysis, there is no statistical significance up to and including 10000 µg a.i./kg diet; Chi² Test [Bonferroni-Holm's corrected, one-sided  $\alpha = 0.05$ ])

chi<sup>2</sup> Test, (Bonferron Holms corrected one sided = 0.05)

n.s. Thean value not statistically significantly different compared to the control

# D. Validity Criteria

D. Validity Criteria Superior Condition of the Internal Representation of the Internal Repres INRA - method for testing posticide toxicity to honeybee brood in laboratory conditions (January 2008) and proposed by the recommendations of the honeybee larvae laboratory ring-test group (AUPINEL et al., 2009) Were not (i.e. mortality in the control group  $\leq 15\%$  and in the reference group  $\geq 50\%$  until day +7) In addition to the validity criteria as proposed by the ring-test group, an additional self-set validity criterion was employed (i.e. mortality in the control group  $\leq 30\%$  until day +22). This self-set validity coterion was applied in order to exclude test runs from which it is difficult to derive biologically meaningful information due to elevated mortality levels.

Table:	Control performance in the individual test runs and associated validity criteria

Validity criteria	Origin of validity criteria	Validity threshold	Obtained results				
			Test run	Test run	Test run 🦠	Test _run	Test ruo
			No. 1	No. 2	No. 3	No. 4	<b>N</b> 6.5
Mortality in the control group until day +7	INRA - method for testing pesticide toxicity to	≤ 15%	14.6%	9.5%	5.424	2.1%	11.9%
Mortality in the reference group until day +7 (Abbott)	honeybee brood in laboratory conditions (January 2008)	≥ 50%	73.2%	81.6%	88.6%	\$3.3% Q	78.4%
Mortality in the control group until day +22	Self-set	≤ 30%	31.3%‡	16.7%	Ø*	18/8% × × × × × × × × × × × × × × × × × × ×	28.6%

Actual control performance at the end of the test has not met the self-selvalidity criterion

# E. Biological Endpoints Derived

Overall, three test runs (2, 4 and 5) fulfilled both, the validity criteria as proposed by the INRA-method (January, 2008) for testing pesticide toxicity to honeyboo brood in laboratory conditions and the self set validity-criterion of ≤ 30% mortality in the control group until day \$\infty\$2. The statistical processing of the data as obtained in the test runs 2, 4% and 5 consistently revealed no statistically significant effects on mortality of exposed honeybee larvae until day \$22 (end of the test, emergence) at concentrations of up to and including 10000 μg BYI (12960 a.i./kg Olet (Chart Test) Bonferroni-Holms Corrected, one-sided, α = 0.05). This conclusion is supported by the findings of the test runs 1 and 3.

All five independent test runs, as performed during the ourse of this in vitro honeybee larvae study, comply with the validity criterious proposed by the INRA-northod January, 2008) for testing pesticide toxicity to honeybee brood in Jaboratory conditions, i.e., until days 7, \le 15% mortality in the control group and  $\geq 50\%$  mortality in the reference group), three independent test runs (test runs 2, 4 and 5) fulfilled both, the validity criters as proposed by the INRA method (January 2008) and the self-set validity criterion (i.e. 30% prortality in the control group until day +22). The analytical determination of BYI 02960 in the expositive diets of the test item treatment group revealed that the actual concentrations were well in line with the nominal concentrations. The statistical processing of the data as obtained in the test cans 2.14 and 3 consistently revealed no statistically significant effects on mortality of exposed hones bee larvae unto day 22 (end of the test, emergence) at concentrations of up to and including 200000 μg BY 202960 Li./kg aret (Chi² Test, Bonferroni-Holms corrected, one-sided, α = 0.05). The outcome of this statistical evaluation is further supported by the findings of the test runs 1 and 3.

Overally, it can be concluded that the No Observed Effect Concentration (NOEC) as determined in this *in-vitro* hopeybee larvae study is ≥ 10000 μg BYI 02960 a.i./kg diet.

#### **IIA 8.16.2** Other/special studies - field studies

# Effect of BYI 02960 on soil litter bag degradation

In this section the effect of BYI 02960 on soil litter bag degradation is described, this study is a registration requirement in Europe, however there is no specific OECD data heading for the study

Report:	KIIA 8.16.2/01; (2011)
Title:	BYI 02960: Effects on soil litter degradation after spray application
Report No:	LRT-SLD-45/11
Document No:	M-413408-01-2
Guidelines:	Guidance Document on the Breakdown of Organic Matter in Litter Bags (OECD Series on Testing and Assessment, Number 56, 2006)
Deviations:	None None Q Q Q Q Q
GLP:	Yes (certified laboratory) . O Y

#### **EXECUTIVE SUMMARY**

This study was designed to evaluate the effect of BYI (2960 or soil litter degradation. The test item was applied as formulated product BY1002960 SL 200 G (Specification No. 102000021 84-01, Batch-ID.: 2010-001067, Sample description: TOX08907-00, analysed content 201 0 1/2 (15.1 % W/w), density: 1.175 g/mL).

The test item was applied twice by spraying. 1st to represent the placeau concentration and 2nd to represent the yearly application rate on six plots on a field in Germany

On June 14, 2010 BYL 2960 DE 2000 was applied at a rate of 100 g a 1/ha to the treatment plots and by careful harrowing the test item was incorporated into the upper 10 cm soil layer to achieve a plateau concentration of 100 µg a P/kg soll dry weight in 0 - 10 cm soll depth. Two days later, untreated summer wheat Pariety Changin", was sown onto all plots. The seed rate was 230 kg/ha. Forty litterbags per plot were buried. On the same day BYI 02060 SL 200 Swas applied at a single rate of 300 g a.i./ha to the treatment plots simulating the cumulative annual use of BYI 02960. This application rate corresponds to 1490 mL test item/ha or \$754 g test jtem/ha.

The degradation of the straw was determined for the time periods of 0-29 days, 0-92 days and 0-92217 days by recording the weight of undegraded straw (after grinding by a ball mill and incineration).

217 days after Durying of litter bags in soil treated with BYI 02960 (150 g BYI 02960/ha for plateau concentration, and 300 g BYI 02960/ha for the annual fate) a difference in straw degradation of 3% was observed which is below the trigger value of 10%

From the results of this study it can be constided that soil residues of BYI 02960 after long term use (including plateau concentration equivalent to a rate of 150 g a.i./ha combined with an annual rate of neather) ha 300 g a.i./ha as spray application) have no influence on organic matter breakdown 217 days after application.

#### **MATERIAL AND METHODS**

#### A. Materials

# 1. Test material Test item: BYI 02960 SL 200 G Type: Formulated product (soluble (liquid) concentrate Chemical state and description: Liquid, clear brown 102000021884-01 Specification No.: Batch No .: 2010-001067 Nominal content of active BYI 02960: 200 g/L substance: ing to certificate Analytical content of active BYI 02960: 17.0% w/a substance: Density: 1.175 g/mL at 20° Stability of test compound: Expiry date: 14.06 acceptable) 2. Test soil Soil nomenclature Study site Particle size distribution (%) according to D clay (< 0.002 mm): silt (0.002 - 0.063 mm): sand (0.063 - 2.0 mm): Soil properties: pH (1 N KCl) Microbial C (mg/kg $C_{org}(\%)$ $C_{anorg}(\%)$ N (%) P (mg/kg dws) CaCO<sub>3</sub> (%) Water folding capacity g H<sub>2</sub>QH00g dws Cation exchange capacity (m/cq/100 g dr History of soil: Plant protection productonot Fertilisers not sed since mmer wheat Crops: design and metho May 20, 2011 2. Design of biol@gical test Six plots in the field Germany were treated with BYL 2960. Six plots served as untreated control plots. The field

A concentration of 100 µg axi./kg soil dry weight was selected for the plateau concentration in the upper 10 cm soil layer. This corresponds to an application rate of 150 g a.i./ha equivalent to 750 mL test item/ha or 877 g test item/ha.

On June 14, 2010 BYI 02960 SL 200 G was applied at a rate of 150 g a.i./ha in a water volume of 300 L/ha to the treatment plots and by careful harrowing, the test item was incorporated into the upper

10 cm soil layer to achieve a plateau concentration of 100 μg a.s./kg soil dry weight in 0 - 10 cm soil depth. On June 16, 2010, untreated summer wheat, variety "Chamsin", was sown with a seed rate of 230 kg/ha onto all plots and 40 litterbags per plot were buried. At the same day BYI 02960 SL 200  $\checkmark$ was applied at a single rate of 300 g a.i./ha in a water volume of 300 L/ha to the treatment plots & simulating the cumulative annual use of BYI 02960. This application rate corresponds to 1490 on L item/ha or 1754 g test item/ha.

Litterbags (Polyester, 22 x 12 cm, mesh size 8 mm) filled with  $4 \pm 0.1$  g dry wheat straw. Were purchased from closed with a cable tie. A coloured label was fixed on each bag which served as a mark above the so recover the bags after they had been buried.

On June 18, 2010 each treatment plot and control plot was irrigated with about 6 mm water. Top 20, 2010, 3.5 mL precipitation was observed. &

# 3. Observation and measurements

Soil samples were taken on June 14, 2010 directly after application and incorporation of the plate of concentration and on June 21, 2010, 5 day's after application of the annual rate. The degrapation of the straw was determined for the time periods of 9 - 29 days, 0 92 days and 0 - 21 days by recording the weight of undegraded straw (after guinding by a hall mill and incineration). Day 0 was set on June 16, 2010 when litterbags had been buried. Colculating the difference of the weight of the weight of the start of the experiment and the remaining weight at sampling time allowed determination of the degree of degradation.

#### 4. Statistical analysis

In order to determine whether the results reseal statistically significant differences, the weight (in gram) of degraded-straw of the two variants (untreated control and togatments with BYI 02960 SL 200 G) were analysed with the program Tox Rat Pro, Version 2.09. Cochran s test was conducted to test for homogeneity of variances and Kolmogorov Smirnow Test was conducted to test for normal distribution. If the data were normally distributed and homogeneity of variance was given, a Student-t test (two saided,  $\alpha = 0.05$ ) for homogeneous variances was performed. If data were not normally distributed, or if homogeneity of variance was not given the test was repeated with transformed data. If the transformed data too were not normally distributed or homogeneity of variance was not given the A. Weather conditions

Rainfall and temperature during the study are depicted in the following table: non-parametric Mann & Whitney Pair wise Litest (Wilcoxon rank sum test, two sided,  $\alpha = 0.05$ ) was

Table: Monthly Precipitation and average temperature during test period

Month (2010)	Monthly precipitation	Monthly average temperature (°C)
	(mm)	2 m above ground
June	12.3	18.01
July	88.0	21.15
August	203.0	1 (3)
September	109.0	19.5/
October	60.6	6.20 - 1.63
November	129.0	6.20
December	67.5	
January 2011	113.9	4.5
Sum June 2010 to January 2011	783.2 <sub>4</sub>	
		4.5 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4
B. Analytical results	~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	ded by the EPFES gordeline (50% to
Findings are well in agreement with	th analytical Pmits & specifi	led by the FPFES goldeline (50% to

# **B.** Analytical results

Findings are well in agreement with analytical mits, a specified by the EPFES goldeline (50% to 150% of the nominal concentration should be reached) thereby confirming the application of the test substance. The limit of quantification (LQQ) was \$\frac{1}{2} \mu g \text{kg} dry weight soil.

Nominal and analytically verified amounts of BYI 02960 Table:

Nominal application rate  Nominal application rate
[ga.i./ha] *
Plateau concentration
Cumulative annual application 2 200 2 300
$ \Sigma $
Apalysed concentrations
Σ Σ [μg að/kg dry soil]  % Prominal amount
Plateau concentration
[soil samples taken after incorporation]   \( \forall \)
Plateau concentration Cumulative
Plateau concentration Cumulative annual application soil samples taken six days after application
days after application] 💸 🛴 💛 🐧 💸 💍 🕲

<sup>\*</sup> Conversion from µg a.i./kg soil to ga.i./ha and very versa assume a soil depth of 10 cm and a soil density of 1.5 g/mL

# C. Biological Finding

The results of this study howed no statistically significant difference in proportion of straw degradation between untreated control plots and the plots treated with BYI 02960 SL 200 G for of litter-bags 29 and 217 days after petroduction into the Soil.

02960 SL 200 G op organic matter degradation

Means of 4 plots	Éøntro E	BYC02960 \$200 G	% of Control 1)	% Effect <sup>2)</sup>
0 – 29 d* g straw degraded		Q 0.86	92	8
% straw degraded	© 3.37 \( \tilde{\tilde{\tilde{3}}}	21.56		
0 – 92				
g strawdegraded	2,02	2.51**	118	-18
% straw degraded	\$ <b>2</b> .95	62.73		
<b>2</b> – 217				
g straw d@raded	3.06	2.96	97	3
% straw degraded	76.38	74.06		

- \* day 0 was set on June 16, 2010, when the litterbags had been buried
- \*\* statistically significant difference to the control (student-t test,  $\alpha = 0.05$ , two sided)
- 1) corresponding to degraded straw in g, formula: (mass loss treatment\*100)/mass loss control
- 2) corresponding to degraded straw in g, formula: ((mass loss control-mass loss treatment)/mass loss control 00

# D. Validity Criteria

A degradation of  $\geq 60$  % straw in untreated control was reached after 217 days after introduction of litterbags into soil. A coefficient of variation of  $\leq 40$  % for soil litter degradation for the data generated within 217 days in the control plots of the study was achieved (20.72 % 2.25 % and 7.24 % after 29.92 and 217 days, respectively).

Thus the two validity criteria were fulfilled as recommended by the  $\widetilde{Q}$  ECD guideline. The study was terminated since degradation of straw was  $\geq 60$  % in the untreated control after  $\widetilde{Q}$  7 days.

# E. Biological endpoint

217 days after burying of litter bags in soil treated with BY 62966 (150 gBYI 02960/ha for plateau 4 concentration and 300 g BYI 02960/ha for the annual rate) a difference in straw degradation of 3 was observed, which is below the trigger value of 10%.

#### **CONCLUSION**

From the results of this study it can be concluded that soil residues of BYI 02060 after long term use (including plateau concentration equivalent to a rate of 150 g a.i./ha combined with an annual rate of 300 g a.i./ha) have no influence on organic matter breakdown 21 days after application.

Report:	KIIA 8.16.2/02; (2001) &
Title:	BY 92960 Effects on soil bitter degradation if applied as seed treatment
Report No:	D LANT-SLD-46/11 > 2 2 2
Document No:	M-413416-01-27
Guidelines:	OECD No. 56, 2006 OECD Series on Testing and Assessment)
Deviations:	None A & O O
GLP:	Ses (certified laboratory)

#### EXECUTIVE SUMMARY

This study was designed to evaluate the effect of BXV 02960 on soil litter degradation. The test item was applied as formulated product BYI 02960 SL 200 & (Specification No.: 102000021884-01, Batch-ID.: 2010-001067, Sample description: TOX 08907-00, analysed content: 201.0 g/L (17.1% w/w), density: \$175 g/mL) and BYI 02960 FS 480 G (Specification No.: 102000022677-01, Batch-ID.: 2010-001101, sample description: TOX 08940-00, analysed content: 481.4 g/L (40.4% w/w), density: 1.191 g/mL.

The test item was applied twise. First by spraying, represent the plateau concentration (as would occur after multi-year use) and second, as seed featment to represent the annual application rate. The study was performed on six plots on a field in Germany (

On June 14, 2000 BY 12960 SL 200 G was applied at a rate of 150 g a.i./ha to the treatment plots and by careful harrowing, the last item was incorporated into the upper 10 cm soil layer to achieve a plateau concentration of 100 µg a.i./kg soil dry weight in 0 - 10 cm soil depth. Two days later, summer wheat, variety "Chamsin", treated with BYI 02960 FS 480 G was sown onto treatment plots. The seed rate of

230 kg/ha leaded to an application rate of 265 g BYI 02960/ha (nominal). A degree of loading of 109.7 % was reached with the analysed content of 115.37 g a.i./100 kg seeds.

Litter bags were buried in six treatment plots and six untreated plots, respectively, and the degradation of the straw was observed for a period of 217 days. Results obtained from the untreated plots were compared with those of the treatment plots.

The degradation of the straw was determined for the time periods of 0-29 days, 0-92 days and 0 - 217 days by recording the weight of undegraded straw (after grinding by a ball mill and incineration). 217 days after burying of litter bags in soil treated with BY 02960 SL 200 G (150 g BYI 02960/ha for plateau concentration) and the annual rate in form of treated summer wheat seed (nominally 265 g BYI 02960/ha), a difference in graw degradation of 5% compared to the untreated control was observed, which is below the frigger value of 10%. Therefore, it can be concluded that soil residues of BYI 02960 after long term use (including plateau concentration equivalent to a rate of 150 g a.i./ha combined with an annual rate of 265 g a.i./ha as seed treatment) have not influence on organic matter breakdown 217 days after application.

#### MATERIAL AND METHODS

#### A. Materials

# 1. Test materials

Toot	item	1
1 621	пеш	

(spray for plateau concentration):

By 02960 SL 200 G Formulated product (soluble (liquid) concentra

Chemical state and description Liquid, clear Frown Specification No.: Batch No .:

Sample description: 
Nominal confort of active substance: Sample description: BYL02960

.0 Laccording to certificate of Analytical content of active

substance. Density.

Stability of test compound Expiry date 14.06.2012, when stored at 25  $\pm$  5°C

C to 30°C@re also acceptable)

Test item 2

(used as seed thatment) BŶ¥02960¥FS 480.G

Formulation (Flowable concentrate for seed treatment) Type:

Chemical state and description: Beige suspension 102000022677-01 Specification No.: Batch No.: 2610-001101 TŎX **@\$**940-00 Sample description: BYI-02960: 480 g/L Nominal content of active substance

Analytical content of activ BMJ 02960: 481.4 g/L substance:

1.191 g/L

Expiry date: 12.03.2012, when stored at 25  $\pm$  5°C

 $(+2^{\circ}\text{C to } +30^{\circ}\text{C are also acceptable})$ 

Seed dressing: Cereal: Summer wheat 'Chamsin' Dosage: 219 mL BYI 02960 FS 480/dt Nominal content of active substance: 105.12 g a.i./100 kg seeds Analytical content of active 115.37 g a.i./100 kg seeds substance: 109.7 % Degree of loading: Stability of test compound: Approved until 04.11.2010, when stored at 25  $\pm$  5 (+2°C to +30°C are also acceptable) 2. Test soil Soil nomenclature: Study site: Particle size distribution (%) according to DIN: Clay (< 0.002 mm): Silt (0.002 - 0.063 mm): Sand (0.063 - 2.0 mm): Soil properties: pH (1 N KCl) Microbial C (mg/kg dws)  $C_{org}(\%)$  $C_{anorg}(\%)$ N (%) P (mg/kg dws) CaCO<sub>3</sub> (%) Water holding capacity (g H<sub>2</sub>O/400g dws): Cation exchange capacity (may 100 gdry History of soil: Plant protection products not used since Fertiliser not used since Crops: B. Study design and meta 1. In life dates 2. Design of biological Six plots in the field Germany were treated with SYI 02960 fromulated as BOI 02960 applied as SL 200 G (Specification

Germany were treated with BYI 02960 formulated as BØI 02960 applied as SL 200 G (Specification No.: 102000021884-01, Batch-ID.: 2010-001067, sample description: TOX 08907-00, analysed content: 201 g/L (17 10 w/w), density: 1.175 g/m/Q approved until June 14, 2012) and BYI 02960 FS 480 G (Specification No.: 102000022677-01 Batch-ID.: 2010-001101, sample description: TOX 08940-00, analysed content: 4814 g/L (40.4% w/w), density: 1.191 g/mL, approved until March 12, 2012).

A concentration of 100 μga i./kg soil dry weight was selected for the plateau concentration in the upper 10 cm soil layer. This corresponds to an application rate of 150 g a.i./ha equivalent to 750 mL test item/ha or 877 g test. item/ha six plots served as untreated control plots.

On June 14,2010 BYI 02960 SL 200 G was applied at a rate of 150 g a.i./ha in a water volume of 300 L/ha of the treatment plots. On June 16, 2010, untreated summer wheat, variety "Chamsin", was sown with a seed rate of 230 kg/ha onto the control plots. Also on June 16, 2010, dressed summer wheat, variety "Chamsin" treated with BYI 02960 FS 480 G was sown onto the treatment plots (TRE 1-

6). After sowing 40 litterbags per plot were buried. Litterbags (Polyester, 22 x 12 cm, mesh size 8 mm) filled with  $4 \pm 0.1$  g dry wheat straw, had been purchased from

. The bags were closed with a cable tie. A coloured label was fixed on each bag which served as a mark above the soil to recover the bags after they had been buried.

#### 3. Observation and measurements

Soil samples were taken on June 14, 2010, directly after application and incorporation of the plateau concentration and on June 21, 2010, 5 days after application of the annual rate. The degradation of the straw was determined for the time periods of 0-29 days, 9-92 days and 0-217 day by recording the weight of undegraded straw (after grinding by a ball mill and incineration). Day 0 was set on June 16, 2010 when litterbags had been buried. Calculating the difference of the weight of straw at the start of the experiment and the remaining weight at sampling time allowed determination of the degree of degradation.

#### 4. Statistical analysis

In order to determine whether the results reveal statistically significant differences, the weight (in sum) of degraded-straw of the two variants (untreated control and weathers with BYI \$2960) were analysed with the program ToxRatPro, Version 2.09 from ToxRat Solution Gmbbs,

Cochran's test was conducted to test for homogeneity of variances and Kolmogorov-Smirnow Test was conducted to test for normal distribution. If the dark were normally distributed and homogeneity of variance was given, a Student-t test (two aded,  $\omega = 0.05$ ) for homogeneous variances was performed. If data were not normally distributed, or  $\omega$  homogeneity of variance was not given the test was repeated with transformed data if the transformed data too were not normally distributed or homogeneity of variance was not given the non-parametric Mann & Whitney Pair-wise U-test (Wilcoxon rank sum test, two sided,  $\alpha = 0.05$ ) was chosen.

#### RESULTS AND DISCUSSION

#### A. Weather conditions

Rainfall and temperature during the study are depicted in the following table:

Table: Monthly Precipitation and average temperature during test period

Month (2010)		Monthly precipitation (mm)	Monthly average temperature (°C) 2 m above ground
June 4		2 2.3	18.01
July		© \$88.0 ×	21.15
August		2026	17.05
September	\$ . \$ . \$ . \$ . \$ . \$ . \$ . \$ . \$ . \$ .	<i>Ŵ</i> 1 <i>⋔</i> % 0	13.57
October		60.6	10.01
November @		<b>2</b> 129.0	6.20
December		67.5	- 1.63
January 2011		113.9	4.5
Sum June 2000 to Ja	muary 2011 🔊	* 783.2	-

## B. Analytical findings

Findings are well in agreement with analytical limits as specified by the EPFES guideline (50% to 150% of the nominal concentration should be reached) thereby confirming the application of the test substance. The limit of quantification (LOQ) was 5  $\mu$ g/kg dry weight soil.

#### Table: Nominal and analytically verified amounts of BYI 02960

	Nominal application rate		
	[µg a.i./kg dry soil]	[g a.i./ha] *	
Plateau concentration	100	150	
Cumulative annual application	177	265	
Σ	277	415	
	Analysed con	centrations	
	[µg a.i./kg dry soil]	of nominal aprount	
Plateau concentration [soil samples taken after incorporation]	94 💸	945	
Plateau concentration + Cumulative annual application [soil samples taken six days after application]			

<sup>\*</sup> All conversions from µg a.i./kg soil to g a.i./ha and vice versa assume a soil depth of 10 cm and a soil depsity of 1.5 g/mL

#### C. Biological findings

Soil litter degradation is presented in the following table

Table: Effects of BYI 02960 SL 200 6 and BYI 02960 FS 480 G on organic matter degradation

Means of 4 plots	Control @ &	BYI 02960 % of Control 1 % Effect 2)
0 - 29 d*		
g straw degraded	0.93	0 3 3 7 7 7 7 7 9 0 0 0
% straw degraded	23.3%	\$23.37\$\tag{\tag{\tag{\tag{\tag{\tag{\tag{
0 – 92 d*	4, 5 0	1 0 × 1 × 1 × 1
g straw degraded	2Q <sup>2</sup>	2,09
% straw degraded	\$2.95 ( )	\$2.35
0 – 217 d*	, 10, 13, i	3 20 1050 -5
g straw degraded	3.06	3,20 105 -5
% straw degraded	<b>26.38 C</b>	80/08

<sup>\*</sup> day 0 was so on June 16, 2010, when the lifterbags Fad be buried

No statistically significant difference in proportion of straw degradation was observed between untreated control plots and the plots treated with BYI 02960.

#### D. Validity Criteria

A degradation of  $\geq$  60% straw in unit reated control was reached after 217 days after introduction of litter bags into soil. A coefficient of variation of  $\geq$  40% for soil litter degradation for the data generated within the 217 days in the control plot of the study was achieved (20.72%, 8.25% and 7.24% after 29, 92 and 217 days, respectively).

Thus the two validity criteria were fulfitted as recommended by the OECD guideline. The study was terminated since degradation of straw was  $\geq 60\%$  in the untreated control after 217 days.

<sup>1)</sup> Corresponding to degraded stray in g, formula: (mass loss treatment\*100)/mass loss control

<sup>2)</sup> Corressonding to degraded straw in g, formula: ((masoloss control-mass loss treatment)/mass loss control)\*100

#### E. Biological endpoint

CONCLUSION

It can be concluded, that soil residues of BY102960 after long term use (including plateau). Concentration equivalent to a rate of 150 g a.i./ha combined, with an annual/mate of 265 g yri./hasqk seed treatment) have no influence on organic matter breakdown 217 days aftigrapplication. 217 days after burying of litter bags in soil treated with BYI 02960 (150 g BYI 02960/ha for plateau

#### IIA 8.17 Summary and evaluation of points IIA 7 and IIA 8.1 to 8.16

#### **Summary of Point IIA 7, Environmental Fate**

The route of degradation of BYI 02960 in aerobic soil has been determined in European and American soils with four different label positions under standard laboratory conditions at 20°C for 120 days.

Under aerobic conditions two major metabolites were observed, DFA (maximum 33.9%) and 6-CNA (maximum 17.1%). In all label positions there was significant mineralization to <sup>14</sup>CO<sub>2</sub> (maximum ca. 59%) with relatively low formation of non-extractable residues (max. ca. 34%). The results indicate that BYI 02960 is degraded in aerobic soil by microbial activity with an overall mean DT 50 (trigger value) of 73 days.

Under anaerobic soil conditions BYI 02960 was stable and it was concluded that photolysis on the soil surface would not be a significant route of degradation

For BYI 02960 in standard batch equilibrium studies on 6 soils the adsorption  $K_{oc}$  ranged from 4.9 to 132.2 mL/g, desorption  $K_{doc}$  were higher indicating significant stronger sorption. In time dependent sorption studies the sorption of BYI 02960 was shown to increase over time with at ageing factor of 2.4 to 4.4. The  $K_{oc}$  of the major metabolite 6-CNA was determined in four soils (excluding one soil with very low organic carbon content and the sediment) ranged from 10 to 29 indicating medium mobility. The  $K_{oc}$  ads for the metabolite DFA determined in five soils ranged from 1.700 9.5 indicating high mobility in soil.

The hydrolysis study of BY 202960 in steple buffer solutions of pH 47 and 9 showed that the active substance is hydrolytically stable under environment conditions. Photolytically BYI 02960 degraded very rapidly in sterile buffer and natural water studies, based BY 02960 should degrade with a DT 50 of less than a week, if exposed to sunlight. The major degradates were identified as BYI 02960-succinamide (found at max. 39.6% of applied) and BYI 02960-azabrcyclosuccinamide (found at max. 25.9% of applied).

BYI 02960 is regarded as stable under anaerobio aquatic condition, and no major metabolites were formed.

The aerobic biotransformation of BY1 02960 was studied in two water-sediment systems, for a maximum of 120 days in the darkness at 20°C. The test item was applied with three radiolabels per test system. Dissipation of BYI 02960 from the vater phase was mainly characterized by rapid partitioning into the sediment where it is slowly degraded and mineralized. DFA (difluoroacetic acid) was observed as a degradation product. In the vater phases DFA accounted for up to 6.0%, in the sediment extracts for max. 6.9% of the applied radioactivity. No further significant degradation products were observed in the studies except mineralization to carbon dioxide (max. 8.5% of applied) and formation of NER (max. 26.6% of applied). The DF<sub>50</sub> value for BYI 02960 in the entire water/sediment systems was in the range of 193 to 285 days.

In a supportive study the face of BYI 02960 was investigated in pond water and sediment in outdoor microcospie as are equatic model ecosystem for lentic aquatic freshwater systems with different trophic levels. The discipation of BYI 02960 from the supernatant water phase with a mean of 81 days was caused by translocation into the sediment and by degradation. The overall degradation (mean of 95 days) was daster under the prevailing outdoor conditions compared to the standardized laboratory water sediment studies considering the rapid degradation due to photolysis this may due to the enhanced degradation due to sunlight under the outdoor test conditions.

In a further water-sediment study the degradation behavior of DFA applied as test item was investigated. Mineralization to carbon dioxide (max. 25.1% of applied) and formation of NER (max.

investigated. I	Mineralization to ca	arbon dioxide (max. 25.1% of a	applied) and formation of NER (max.
15.8% of appl	ied) was measured	during the study period.	ipplied) and formation of NER (max.
Summary of			
In the following	ng, the endpoints of	f the active ingredient resulting	from the ecotoricological studies are
summarised.		C	
Endpoints for 1	Birds		from the ecotomicological studies are
Test species	Test design	Ecotoxicological endpoint	Reservence & S &
BYI 02960			
Colinus virginianus	acute, oral	LD <sub>50</sub> 232 mg & kg bw	(2019) M-386036-04-1, KAIA 8.1, 1401
Serinus canaria	acute, oral	LD <sub>50</sub> 330 mg a.i kg bw	% 408.5% -01-1, KIIA & 1.1/02 \\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \
Gallus gallus domesticus	acute, oral	LD <sub>50</sub> <b>2000 mg a.i./kg</b> bw	M-4Q0519-UF-2, K*MA 8.1, 1/03
Anas platyrhynchos	5-day-feeding	LC <sub>50</sub> >4741 mg ai kg diet ≡	(2010)
Colinus virginianus	5-day-feeding	LC <sub>50</sub> >4876 mg a⁄a/kg diet = \$770 mg a.i./kg bw/d NOEL \$133 may a.i./kg diet = \$170 mg a.i./kg bw/d.	& (2000) M-394335-01-1 KII-08.1.2/02
Anas platyrhynchos	20-week feeding chronic reproduction	NQÆL ≥845 mg/a.i./kg/diet ≥81 mg/a.i./kg/bw/d	& (2011), M-412917-02¥1,KIIA 8.1.4/01
Colinus virginianus	23-week feeding chronic, production	NOAEL 302 mg a.l./kg diêt = 040 mg a.i./kg bw/d	(2012), M-424704-01-1, KIIA 8.1.4/02

# Endpoints for Aquatic organisms. Freshwater Fish

~ ~ ~			
Test species	Test design 🦑	Ecotoxicological endpoint	Reference
		Umg sa ji./L] Ju	
Oncorhynchus	acute, 96 Q	LC <sub>0</sub> > 7 * .2 (mth) <sup>2</sup>	& (2010)
mykiss ~			M-390611-01-1, KIIA 8.2.1.1/01
Pimephales 4	acute, 96 h	$C_{50} \Leftrightarrow 70.5 \text{ (mm)}$ $C_{50} \Leftrightarrow 70.5 \text{ (mm)}$	& (2010)
promelas 🔊		NOE 270,5 (mms)	M-392560-01-1, KIIA 8.2.1.2/01
Cyprinus 🛇	acute, 96 h	$LC_{50} \approx 700 \text{ (mm)}$	(2011)
carpio <<		NOEC 4.41@mm)	M-420407-01-2, KIIA 8.2.1.2/02
Pimephales	early life stage	NOEC 4.41 (mm)	& (2011)
promelas	L(H0(aS) 35d ≪	LOPE 8.6 (mm)	M-409339-01-1, KIIA 8.2.4/01
mm = mean me	aswred A	$\psi_{_{\mathbf{A}}}$	
mm = mean me	(RAS), 35d		
8			

#### **Endpoints for Aquatic organisms: Invertebrates**

Daphnia   acute, 48 h	Test species	Test design	Ecotoxicological endpoint	Reference
Daphnia   acute, 48 h   EC <sub>50</sub>   > 77.6 (mm)   M-357476-01-1, KIIA 8.3.1.1/04     Daphnia   chronic, static   NOEC   3.2 (nom)   M-414066-01-2, KIIA 8.3.1.1/04     Chironomus   acute, 48 h   EC <sub>50</sub>   0.062 (nom)   M-414736-01-1, KIIA 8.3.2.4/01     Chironomus   chronic, spiked   NOEC   0.025 (nom)   M-414736-01-1, KIIA 8.3.1.2/04     Chironomus   chronic, spiked   NOEC   0.0105 (mi)   M-414736-01-1, KIIA 8.3.1.2/04     Chironomus   chronic, spiked   NOEC   0.0213 (mi)   M-414736-01-1, KIIA 8.3.2.2/01     EC <sub>50</sub>   0.0353 (mi)   EC <sub>15</sub>   0.0219 (pi)     BYI 02960-succinamide   Daphnia   chronic, 21 d   NOEC   43.3(nom)   M-24700-01-2, KIIA 8.3.2.1/02     Chironomus   acute, 48 h   EC <sub>50</sub>   M-10 (mi)   M-417386-01-3, KIIA 8.3.1.2/02     BYI 02960-azabicyclosuccinamide   Chironomus   acute, 48 h   EC <sub>50</sub>   M-10 (mi)   M-417386-01-3, KIIA 8.3.1.2/02     BYI 02960-azabicyclosuccinamide   Chironomus   acute, 48 h   EC <sub>50</sub>   M-10 (mi)   M-424404-01-1, KIIA 8.3.1.2/02     Chironomus   acute, 48 h   EC <sub>50</sub>   M-24404-01-1, KIIA 8.3.1.1/02     Daphnia   acute, 48 h   EC <sub>50</sub>   M-24404-01-1, KIIA 8.3.1.1/02     Chironomus   chronic, spiked   NOEC   M-10 (mi)   M-415913-01-2, KIIA 8.3.1.1/02     Chironomus   acute, 48 h   EC <sub>50</sub>   M-10 (mi)   M-415913-01-2, KIIA 8.3.1.1/02     Chironomus   acute, 48 h   EC <sub>50</sub>   M-10 (mi)   M-415913-01-2, KIIA 8.3.1.1/02     Chironomus   acute, 48 h   EC <sub>50</sub>   M-10 (mi)   M-415913-01-2, KIIA 8.3.1.1/02     Chironomus   acute, 48 h   EC <sub>50</sub>   M-10 (mi)   M-415913-01-2, KIIA 8.3.1.1/03     Chironomus   acute, 48 h   M-10 (mi)   M-10	DI/I 020 (0		[mg a.i./L]	
Mosc		1		* * *
Daphnia   Chronic, static   NOEC   3.2 (nom)   (2011)   M-414066-01-2, KIIA 8.3.2 (101   M-414066-01-2)   KIIA 8.3.2 (101   M-414066-01-2)   KIIA 8.3.2 (101   M-41473501-1, KIIA 8.3.1.2/01   M-41473501-1, KIIA 8.3.1.2/02   M-41473501-1, KIIA 8.3.1.2/03   M-424404-01-1, KIIA 8.3.1.2/03   M-424404-01-1, KIIA 8.3.1.1/02   M-42404-01-1, KIIA 8.3.1.1/03   M-42404-01-1, KIIA 8.3.1.1/04   M-42404-01-1, KIIA 8.3.1.1/04   M-42404-01-1, KIIA 8.3.1.1/04   M-42404-01-1, KIIA 8.3.1.1/	1	acute, 48 h		
Magna   renewal   LOEC   6.4 (nom)   M-414066-0]-2, KIIA 8.3.2.001   M-41473601-1, KIIA 8.3.1.2/04   M-41473601-1, KIIA 8.3.1.2/			- ( )	
Chironomus   chronic, spiked   water, 28 d   LOEC   0.0105 (mi)   water, 28 d   LOEC   0.0213 (mi)   water, 28 d   LOEC   0.0219 (mi)   water, 28 d   LOEC   0.0219 (mi)   water, 28 d   LOEC   100 (nom)   water, 28 d   LOEC   100 (nom)   water, 28 d   LOEC   100 (nom)   water, 28 d   wat	Daphnia	chronic, static	\ /	
Chironomus   chronic, spiked   NOEC   0.055 (nom)   M-41473&01-1, KIIA 8&1.2/05	magna		\ /	
NOEC   0.025 (nom)   M-414739-01-1, KIIA 8 \( \) 1.2 \( \) 1.2 \( \) 1.2 \( \) 1.2 \( \) 1.3 \( \) 1.3 \( \) 1.3 \( \) 1.4 \( \) 1.4 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \( \) 1.5 \	Chironomus	acute, 48 h		(201)
Chironomus riparius water, 28 d NOEC 0.0105 (mi) LOEC 0.0213 (mi) M-46792-01-2 KIIA 8.3 22/01 EC <sub>50</sub> 0.0353 (mi) EC <sub>15</sub> 0.0219 (mi) M-224700-01-2 KIIA 8.3 22/01 M-224700-01-2 KIIA 8.3 21/02	riparius		NOEC 0.025 (nom)	M-41473 901-1, KIIA 8 3.1.2/96 9
Record   R	Chironomus			(2011)
BYI 02960-succinamide    Daphnia	riparius	water, 28 d	LOEC 0.0213 (mi) <sub>4</sub> <sup>y</sup>	M-400792-01-2
Daphnia   Chronic, 21 d   NOEC   43.3 (nom)   MQ 24700-61-2, KIIA 8.5 2.1/02 (2012)   MQ 24700-61-2, KIIA 8.5 2.1/02 (2011)				KIIÃ8.3.222/01
Chironomus   acute, 48 h   EC <sub>50</sub>   100 (nm)   M-24700-01-2, KIIA 8.52.1/020   M-417-86-01-3, KIIA 8.3.1.2/02   M-417-86-01-3, KIIA 8.3.1.2/02   M-417-86-01-3, KIIA 8.3.1.2/03   M-424-01-1, KIIA 8.3.1.2/03   M-424-01-1, KIIA 8.3.1.1/02   M-415-913-01-2, KIIA 8.3.1.1/02   M-415-913-01-2, KIIA 8.3.1.1/02   M-415-913-01-2, KIIA 8.3.1.1/03   M-1965-90-01-2, KIIA 8.3.1.1/03   M-1965-90-01			EC <sub>15</sub> 0.0219 (201)	
Chironomus   acute, 48 h   EC <sub>50</sub>   100 (nm)   M-24700-01-2, KIIA 8.52.1/020   M-417-86-01-3, KIIA 8.3.1.2/02   M-417-86-01-3, KIIA 8.3.1.2/02   M-417-86-01-3, KIIA 8.3.1.2/03   M-424-01-1, KIIA 8.3.1.2/03   M-424-01-1, KIIA 8.3.1.1/02   M-415-913-01-2, KIIA 8.3.1.1/02   M-415-913-01-2, KIIA 8.3.1.1/02   M-415-913-01-2, KIIA 8.3.1.1/03   M-1965-90-01-2, KIIA 8.3.1.1/03   M-1965-90-01	BYI 02960-su	ccinamide	w. b° ×	
Chironomus   acute, 48 h   EC <sub>50</sub>   100 (mi)   M-41   386-01   3, KII   8.3.1   3/02    BYI 02960-azabicyclosuccinamide   Chironomus   acute, 48 h   EQ <sub>50</sub>   000 (mi)   M-424   64-01   KII   8.3.1.2/03    DFA (tested as Sodium difluoro acetate)   M-40932   6-01-2, KII   8.3.1.1/02    Chironomus   chronic, spiked   NOEC   100 (nom)   M-415913-04-2, KII   8.3.1.1/02    Chironomic   chronic acid (6   NA)   M-415913-04-2, KII   8.3.1.1/03    Chironomus   acute, 48 h   EC <sub>50</sub>   95.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   EC <sub>50</sub>   95.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   EC <sub>50</sub>   95.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   295.1 (nom)   M-1965	Daphnia	chronic, 21 d	NOEC 43.3@nom), @ **	(2012) r
Chironomus   acute, 48 h   EC <sub>50</sub>   100 (mi)   M-41   386-01   3, KII   8.3.1   3/02    BYI 02960-azabicyclosuccinamide   Chironomus   acute, 48 h   EQ <sub>50</sub>   000 (mi)   M-424   64-01   KII   8.3.1.2/03    DFA (tested as Sodium difluoro acetate)   M-40932   6-01-2, KII   8.3.1.1/02    Chironomus   chronic, spiked   NOEC   100 (nom)   M-415913-04-2, KII   8.3.1.1/02    Chironomic   chronic acid (6   NA)   M-415913-04-2, KII   8.3.1.1/03    Chironomus   acute, 48 h   EC <sub>50</sub>   95.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   EC <sub>50</sub>   95.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   EC <sub>50</sub>   95.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 96 h   LC <sub>50</sub>   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   295.1 (nom)   M-196569-01   KII   8.3.1.1/03    Chironomus   acute, 48 h   295.1 (nom)   M-1965	magna		LOEC 100 (nom)	MQ24700-01-2, KIIA 8. 62.1/020 "
Chironomus   acute, 48 h   EQ 30   700 (nm)   1424(04-01   KIIA 8.3.1.2/03    DFA (tested as Sodium difluoro acetate)   16250   100 (nom)   16201   16201   16201    Daphnia   acute, 48 h   EC 50   100 (nom)   16201   16201    Chironomus   chronic, spiked   NOEC   100 (nom)   16201   16201    Chironomus   chronic acid (6 (NA)   16201   16201    Daphnia   acute, 48 h   EQ 50   100 (nom)   16201    Daphnia   acute, 48 h   EQ 50   100 (nom)   16201    Daphnia   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ	Chironomus	acute, 48 h	EC <sub>50</sub> > 100 (m)	(2011)
Chironomus   acute, 48 h   EQ 30   700 (nm)   1424(04-01   KIIA 8.3.1.2/03    DFA (tested as Sodium difluoro acetate)   16250   100 (nom)   16201   16201   16201    Daphnia   acute, 48 h   EC 50   100 (nom)   16201   16201    Chironomus   chronic, spiked   NOEC   100 (nom)   16201   16201    Chironomus   chronic acid (6 (NA)   16201   16201    Daphnia   acute, 48 h   EQ 50   100 (nom)   16201    Daphnia   acute, 48 h   EQ 50   100 (nom)   16201    Daphnia   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ 50   100 (nom)   16201    Chironomus   acute, 48 h   EQ	riparius		NOEC 71 (mai) 0 .	M-41 386-01-8, KII 88.3.1 2402
DFA (tested as Sodium difluoro acetate)    Daphnia   acute, 48 h   EC <sub>50</sub>   10 (nom)   M*409326-01-2 KIIA 8 3.1.1/02	BYI 02960-aza	<u>abicyclosuccinamide</u>		
DFA (tested as Sodium difluoro acetate)    Daphnia   acute, 48 h   EC <sub>50</sub>   10 (nom)   M*409326-01-2 KIIA 8 3.1.1/02	Chironomus	acute, 48 h	EQ50 >000 (min) V	(2007) 5 5 5
Daphnia magna         acute, 48 h         EC <sub>50</sub> → 10 (nom) magna         M20EC (10 (nom) magna         M2011 (2011) magna         M2011 (201	riparius		TWOLCE / I (III)	M-42464-01-KIIA-3.3.1.2493
Magna	DFA (tested a	s Sodium difluoro aç	giate) 📞 🛷 🤝 🦪	
Chironomus chronic, spiked water, 28 d	Daphnia	acute, 48 h	$EC_{50}$ > 10 (nom)	
6-chloronicotinic acid (6 (NA)  Daphnia acute, 78 h  OEC 95.1 (mm)  M-196569-0124, KIIA 8.3.1.1/03  Chironomus tentans  Chironomus Chironomus Chronomus Chro	magna		NOEC (nom)	
6-chloronicotinic acid (6 (NA)  Daphnia acute, 78 h  OEC 95.1 (mm)  M-196569-0124, KIIA 8.3.1.1/03  Chironomus tentans  Chironomus Chironomus Chronomus Chro	Chironomus	chronic, spiked	NOEC 100 (nom)	
Daphnia       acute, 28 h       \$\sqrt{0}_{50}\$ \$\sqrt{95.1 (hrm)}\$ \$\sqrt{0}\$ \$\sq	riparius			M-415913-64-2, KJA 8.3.2.2/02
Chironomus chronic spiked NOEC $\geq 100$ (som) $\geq 100$ (2011)				
Chironomus chronic spiked NOEC $\geq 100$ (som) $\geq 100$ (2011)	Daphnia	acute, A8 h	EC <sub>50</sub> 295.1 (mm)	
Chironomus chronic spiked NOEC $\geq 100$ (som) $\geq 100$ (2011)	magna		NOEC 95.1 (mm) Q	M-196569-01@1, KIIA 8.3.1.1/03
Chironomus chronic spiked NOEC $\geq 100$ (som) $\geq 100$ (2011)	Chironomus	acotte, 96 h	LCso >1 (mi)	
Chironomus chronic spiked NOEC $\geq 100$ (som) $\geq 100$ (2011)	tentans		NOEC (Mi) V	№048448-01-1, KIIA 8.3.1.2/04
riparius water, 28 dw water, 28	Chironomus <sub>E</sub>		$ NOEC_a  \ge 100 \text{ (point)}$	(2011)
	riparius 🦠	water, 28 de		M-416604-02-2, KIIA 8.3.2.2/03

mm = mean measured concentration; non = nominal concentration; oi = initial measured concentration

## Endpoints for Aquatic organisms; Marine organisms

<b>Test species</b>	Test desten	Ecotoxicological endpoine	Reference
^		Ymg a.j. [L]	
Cyprinodon A	static acute, 96 h	$LC_{50} > 83.9 \text{ (mm)}$	& (2009)
variegatus		NOEC 83.9 (mm)	M-357479-01-1, KIIA 8.11.1/01
Crassostrea	acute, Now-	EC‰ 29 (mm) NOEC⊘≥ 29 (mm)	,
virginica	through, 96 kg	NOECQ 29 (mm)	(2009), M-361668-01-1, KIIA 8.11.1/02
Americamysis	static acute, 96 h	EC <sub>5</sub> 0.26 (mm)	, & (2009),
bahia	L 4 2'	NOEC 0:22 (mm)	M-364620-01-1, KIIA 8.11.1/03
Americamysis	Pife cýcle, flox	NOEC Ø.0132 (mm)	, (2011), M-
bahia 🛴	through, 2800 ×	LOEC♥ 0.0236 (mm)	420783-01-1, KIIA 8.11.1/04

mm = mean measured

### **Endpoints for Aquatic organisms: Algae and Aquatic plants**

Test species	Test design	Ecotoxicological endpoint	Reference
		[mg a.i./L]	
BYI 02960			
Pseudokirchneriella subcapitata	growth inhibition, 96 h	$ \begin{array}{ll} E_r C_{50} &> 80 \; (nom) \\ NOE_r C &\geq 80 \; (nom) \end{array} $	& (2010) M-397552-012, KIIA 8.4/01
Lemna gibba	growth inhibition, 7 d	$\begin{array}{l} E_b C_{50 \; (frond \; no.)} > 67.7 \; (mm) \\ E_r C_{50 \; (frond \; no)} > 67.7 \; (mm) \\ NOEC_{(frond \; no)}  34.2 \; (com) \end{array}$	(2010), M-398376-01-1- KIIA 86/01
BYI 02960-succinar	nide	<b>, %</b> ,	
Pseudokirchneriella subcapitata	growth inhibition test, 72 h	$E_rC_{50} > 10 \text{ (norm)}$ $NOE_rC \geq 10 \text{ (norm)}$	(2011), M-414020-01-20 (2011), M-414020-01-20 (2011)
DFA (tested as Sodi	um difluoro acetate		
Pseudokirchneriella subcapitata	growth inhibition test, 72 h	E <sub>r</sub> C <sub>50</sub> > 10 (nom) NOE <sub>r</sub> C (nom)	(2010); M-409118-01-2 KUA 8.4/02
6-chloronicotinic ac	id (6-CNA)		
Pseudokirchneriella subcapitata	test, 72 h	ErGs > 100 (nom)	(2013), M-424145-01-2 KHX 8.4/04
A E <sub>r</sub> C <sub>50</sub> at a test conce	entration of 100 mg p	A./L, pH/adjusted (pH/2.5 - 8	
Endpoints for Aqua	Q	nibijas 8 5	KHA 8.4/04 C
Test species	Test design	Ecoroxicological endpoin	Reference

Test species	Test design	Ecoroxicological endpoint Reference
BYI 02960		
Xenopus laevis		$LC_{50}$ $> 73$ $mm$ $0$ $4$ $8.2.1.1/02$ $M-4/7822-0/7-1, KIIA 8.2.1.1/02$

# Endpoints for Honeybees: Acute contact and oral toxicity test

Test species	Test design	<b>Ecotoxicologica</b>	lendpoint 0	Reference
BYI 02960 🙋		A	Ti a, Ti	
Apis mellifera	Acute contact &	LD <sub>50 contact</sub> , 96 L	122. μg an./bee	& (2008)
	oral V	LD Coral, 48h	1.2 μg a/bee	M-308904-02-1, KIIA 8.7.1/01
Difluoro-ethyl-	amino@uranohe (DEEAI			
Apis mellifera	Agute contact & U	D <sub>50</sub> contact, 48 h	>100 a.i./bee	, 2010
	oral Q" S	LD50 % 48 h	>8 <b>1</b>	M-398557-01-2, KIIA 8.7.1/02
BYI 02960-hyd	ODXY CONTRACTOR		<u> </u>	
Apis mellifera	Acute contact &	Log 50 contact, 48 h	100 μg a.i./bee	, 2011
	oral limit test,	DED 50 of all 48 h	>105.3 µg a.i./bee	M-409606-01-2, KIIA 8.7.1/03
Difluoroacetic :	acid (DFA) 🧪 🤍			
Apis mellifera	Acute contact & @	LIM contact 18 h	>100 μg a.i./bee	, 2010
	oral limit	LD 50 ora 8 h	>107.9 μg a.i./bee	M-367915-01-2, KIIA 8.7.1/04
	ic acid (6-CNA) 🎸	<u> </u>		
Apis mellifera	Acute contact &	LD <sub>50</sub> contact, 48 h	>100 μg a.i./bee	, 2010
	oral Vimit test	I 50 oral, 48 h	>107.1 µg a.i./bee	M-395279-01-2, KIIA 8.7.1/05
6-chloropicolyl	alcorol (6-CPA)	¥		
Apis mellifera	Acute contact &	LD <sub>50</sub> contact, 48 h	>100 μg a.i./bee	, 2010
	oral finit test	LD <sub>50 oral, 48 h</sub>	>106.7 µg a.i./bee	M-361234-01-2, KIIA 8.7.1/06
		·	·	

#### Honeybees: Further testing

Test species	Test design	Ecoto	xicological endpoint	Reference
BYI 02960				<u>V</u>
Apis	Chronic effects: 10 d conti	nuous	No adverse effects	, 2010 M-400539-01-2
mellifera	feeding (laboratory), adult		(mortality & behavior);	M-400539-01-2
	honeybees		NOEC = $10000 \mu g a.i./L$	KIIA 806.1/01
Difluoro-ethy	l-amino-furanone (DFEA)	F)		
Apis	Chronic effects: 10 d conti	nuous	No adverse effects	, 2012 M=425174-01-2
mellifera	feeding (laboratory), adult		(mortality & hehavior);	M=425174-01-2,° > ° > ° > ° > ° > ° > ° > ° > ° > ° >
-	honeybees		NOEC = 10000 μg a.i./L	ØKIIA 8.16.1/02,
BYI 02960-h	ydroxy			
Apis	Chronic effects: 10 d conti	nuous	No adverse effects	, 20 (3) Q , Q
mellifera	feeding (laboratory), adult		(mortality & behavior),	M°425242-01-2C
	honeybees		NO(5) = 10000 μg a/i./L	ØKIIA 8.¥6.1/Q©* Ø Ø
Difluoroaceti	c acid (DFA)			
Apis	Chronic effects: 10 d conti	nuous	No advose effects	, 2012
mellifera	feeding (laboratory), adult	4	(mortality & behavior),	M-425105-01-6
	honeybees	Ž,	NOEC = 10000 μg a.f./L 🙎	KIIA\$.16.1/04 & @]
6-chloronicot	inic acid (6-CNA)	a.y		
Apis	Chronic effects: 10 d conti	n@us	No adverse effects &	<b>20</b> 12 <b>3</b>
mellifera	feeding (laboratory), adult		(mortality & behaviol);	9M-425985-01€ .©
	honeybees	, "C	NOEC = 10000 μg a.i./L	M-425\\$55-01\\$ KIIA\\$.16.\\$\\$5
6-СНМР		4.)		
Apis	Chronic effects: 100 conti	nwous 🕍	No adverse effects	2012
mellifera	feeding (laboratory), adult,	Ĉ	(mostality & behavior); >	M-42 <b>9</b> 59-01-2
	honeybees O		NOEC = $10000 \mu g a.i./L$	KUA 8.16 166
		- <del>'</del>	· ^	

Test species	n-targer arthropods  Sest design  Ecotoxicological entipoint  Reference	
BYI 02960	Laboratory@class	
Aphidius Srhopalosiphi Typhlodromas pyr	Laboratory glass plates  Laboratory, glass p	
rhopalosiphi 👸	plates M_366965-01-2, KIIA 8.8.1.	1/01
Typhlodromas pyr	Laboratory, glass $LR_{50}$ 17.3 g a.i./ha (2010)	_ ,
	plates 6 M-366957-01-2, KIIA 8.8.1.2	2/01
6		
4		
, <del>W</del>		
.1		
A.		
a S		
, ( ))		

### **Endpoints for Soil organisms**

Test species	Test design	Ecotoxicological endpoint	Reference
BYI 02960			w i
Eisenia fetida	acute, 14 d	LC <sub>50</sub> 192.9 mg a.i./kg dws	(2010)
	(10% peat in test soil)		M-363742-01-2, KIIA 8.9.1/03
BYI 02960 SL 2	200		
Eisenia fetida	reproduction, 56 d	NOEC 8.9 mg prod./kg dws	<b>2</b> 010)
	(10% peat in test soil)		M-392964-01-2, KIIA 9.9.2/00
Folsomia	chronic, 28 d	NOEC 8.47 mg prod/kg dws	(2009)
candida	(5% peat in test soil)		M&59728-01-2, K∏A 8, 1001 €
Hypoaspis	chronic, 14 d	NOEC ≥1000 mg prod./kg	(2010)
aculeifer	(5% peat in test soil)	dws	M-358752-01©1,KII28.14/Q
Difluoroacetic a	ncid (DFA)		
Eisenia fetida	acute, 14 d	LC <sub>50</sub> >1000 mg p.m./kg dws	(2007)
	(10% peat in test soil)	4. 6° 5°	M-368895-01-2KIIA-8,9.1/02
Eisenia fetida	reproduction, 56 d	NOEC 62.0 fog p.m kg dwg	(2010)
	(10% peat in test soil)		M-398061-01-2, KdVA 8.97/02
Folsomia	chronic, 28 d	NOEC \$100 mg y.m./kg	(201,0)
candida	(5% peat in test soil)	ANS STORY	M-368675-012,KIIA&.14/02
Hypoaspis	chronic, 14 d	NOEC ≥1000 mg p m./kg ≤	(2010)
aculeifer	(5% peat in test soil)	dws V	M-390091301-2,KSTA 8.1405
6-chloronicotini	ic acid (6-CNA) 🔍 🔍		
Eisenia fetida	acute, 14 d	LC50 01000 rug p.m Rg dws	
	(10% peat in test soil)		M-196591-09-1,KKA 8.9.1/03
Eisenia fetida	reproduction, 56 d &	NOGC 95 mg p.m./kg dws	(2011)
	(10% peat inctest son)		M-413562-02-2, KIIA 8.9.2/03
Folsomia	chronic, 28 d	NOEC 90 mg/p.m./kg/dws	(2010)
candida	(5% peat/in test soil)		M407861-01-2, KIIA 8.14/03
Hypoaspis	chronic, 14 d	NOEC 300 mgp.m./kg	, A. (2011)
aculeifer	(5% peat in vest soil)	dws ~ ~ ~	M-40434-01-2, KIIA 8.14/06

dws = dry weight sol

# Endpoints for Organic matter breakdown:

Test species	Test design	Ecoloxicological endpoint	Reference	
BYI 02960 SL 2000	BYI 02960 SL 200G OF OF STANDARD OF STANDA			
Soil litter	2100, 2	Soil treated with 150 ga i./ha for plateau	(2011)	
degradation	spraying 0	concentration + the annual rate of	M-413408-01-2	
(		3000g a.i./ha)	KIIA 8.16.2/01	
	praying 7	No influence O		
degradation  BYI 02960 SL 200	& FS 480			
Soil litter degradation	217 d seed treatment	soil meated with 150 g a.i./ha for plateau	(2011)	
degradation	seed treatment	concentration + the annual rate in form	M-413416-01-2	
×4 n		of treated summer wheat seed	KIIA 8.16.2/02	
<b>S</b>		(265 ga.i./ha)		
	, ,	→ No influence		
4	′ <u> </u>	→ No influence		
		, 2		
Otreated summer wheat seed (265 ga.i./hay  → No influence  KIIA 8.16.2/02				

#### **Endpoints for Soil micro-organisms**

Test species	Test design	Ecotoxicologica	al endpoint	Reference	
BYI 02960				The state of the s	
N-cycle	28 d	no influence	0.3 kg a.i./ha	(2009)	
			3 kg a.i./ha	M-359803-01-1 KIIA 8.10.1/0	
C-cycle	28 d	no influence	0.3 kg a.i./ha	(2011)	
			3 kg a.i./ha	M-417194-01-1, KIIA 8.10-2/01	
6-chloronicotinic	acid				
N-cycle	28 d	no influence	1.0 kg p.m Pa	(2011)	
			1.0 kg p.in.a	M-40\$628-01-1, KIL\$8.10-002	
dws = dry weight s	dws = dry weight soil				
	Non-target terrestrial plants				
Non toward toward	Non-target terrestrial plants				
Non-target terres	triai piants		~ (i		
Test species	Test design	Ecotoxic	Sological@ndpoint	Reference Y Y	
BYI 02960 SL 200					
11 plant species	Vegetative vi	gour No adv	erse@ffects Ø5% (	of Survival $(200)$ $\circ$	

#### Non-target terrestrial plants

Test species	Test design	Ecotoxicological and point Reference &
BYI 02960 SL 200		
11 plant species	Vegetative vigour	No adverse effects 25% of survival, visual playtotoxicity, growth, shoot M-397/734-01-1
	test, application rate	visual playtotoxicity, growth, shoot M-397/734-01-1
	410 g a.i./ha	Wilding and shopedry weight
11 plant species	Seedling emergence	No adverse effects 25% on (2000)
	test, application rate	em@gence.survivatevisuate
	410 g a.i./ha 💝	phytotoxicity, growth, shoot length and KIIA 612/02
	<b></b>	shoot do weight of o c

## Efficacy of metabolite DFA compared to the parent compound

Test species	Test design Ecotoxicological endpoin Reference
Difluoroacetic acid	
Sucking and biting	Microtitet plate In all lest systems it wild be & (2010)
	Test and whole demonstrated that the metabolite DFA 386333-01-1
pepper, cotton and	
cabbage	(greenhouse) Insecticulal efficacy compared to the @
Č	parent compound BY 02960
Sewage treatment	

#### Sewage treatment

	- <del> </del>		
Test species	Dest design		Reference
BYI 02960			
Activated sludge@	Resportation	$\mathcal{L}_{50}$ $\mathcal{O}_{1000}$ $\mathcal{O}_{1000}$ $\mathcal{O}_{1000}$ $\mathcal{O}_{1000}$	(2010)
, <sup>2</sup> Q	inhibition est	Ecotoxicologucal endpoint  EC <sub>50</sub> 1000 ng a.i./V  EC <sub>10</sub> 472.5 mg@ri./L	M-377311-01-1, KIIA 8.15/01
	, , , , , , , , , , , , , , , , , , ,		
√ "			
4			
·	\ \ \( \( \text{\text{\$\pi}} \)		
	′ 4 \ _O~		
Ŏ,			
		4 Q	
Ŭ <i>j</i>		<b>*</b>	
79 D	A. Q		
, Q			
Ö			



## List of BYI 02960 metabolites included in this section

In the original study reports on BYI 02960 the metabolites are sometimes named by different synonyms, the metabolites referred to in this section are summarized below. Full details are provided in Document N.

Jocum	ent N.		Occurrence  All matrices  All matrices
	Name, Structure	Molecular formula	Occurrence  all matrices
	IUPAC name	molar mass	
	CAS name, [CAS number]	Other names / codes	
a.s.	BYF 02960 (parent compound)	(%	full matrices
<b>u</b> .5.	O (parent compound)	$C_{12}$ $H_{11}$ $Cl$ $F_2$ $N_2$ $O_2$	Full matrices D
	Ĭ	200 60 2/201 8	fall matrices
		288.68 g/mol 🗸	
		Flupyradifurone	
	N		
	F		
M03	BYI 02960-OH		
	Q	288.68 g/mol Flupyradifurene F	Animal, Plant:
		304 & g/mot ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	
		BVD02960-Rydroxy	
		BCS CO7/36/	D 8 5 . V
	N ON ON	304 68 g/mov BY 02960 hydroxy BCS-CQ74364	Animal, Plani:
		Y	
	CI N		
	Г 💥		
M21	BYI 02960-CHMP	C6 H6 Cl N Ø  143.57 g/mor  6 PA  6 Chloro-pico placohor  BCS AA52175	
	OH JOH	C6 H6 Cl N Q	Plänt:
	QH Q	143.57 g/mol	Lant.
		143.37 g/1491	
	CI N J J J J J J J J J J J J J J J J J J	6 PA S	<b>Y</b>
		(b-chloro-piçolylacohof)	
		BC\$-AA52175 ~	
		6-chloro-picotylacohol) BCS-AA52175	
M27	6-CNA &		
	6-CNA OF THE PARTY	HC6 H4 OHN O2 O	Animal, Plant:
		157.56 g/me	Environment
		6-chloropicotinic acid	Aerobic soil (major)
		10-0 (in reports from Namon	Tieroore son (major)
	CI N S	Lodo Co I td	
		DVE 02060 @ CNA	
		D 11%2900°0°CINA	
M34	BYI 02960-difluoro ayl-amino-fu	r 00 ne	i
		$\mathbb{F}_{C_6} H_2 \mathbb{F}_2 N \mathbb{Q}_2$	Animal, Plant
		163\$72 g/mor	
		DE EAF	
Ž,		O. ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	
V	THE HOTE OF A	, °O,	
1111	DFA JF JF		l
M44	DIA A A A	<b>V</b>	LAudinial Dlay
		<b>Q</b> H <sub>2</sub> F <sub>2</sub> O <sub>2</sub>	Animal, Plant:
	LAST OF POST	96.03 g/mol	Environment
	THE ST. O	difluroacetic acid	Aerobic Soil (major)
		BYI 02960-DFA	Aerobic water/Sediment (major)
6	Ų Õ'F Ď' ¸Ž		
		BCS-AA56716	
4		(In aquatic studies, tested as	
		sodium difluoroacetate (Na-salt	
		of difluoroacetic acid) (code:	
		BCS-AB60481)	

1	Nama Stanatura	Molocular former-1	Occurrence
	Name, Structure	Molecular formula	Occurrence
	IUPAC name	molar mass	
	CAS name, [CAS number]	Other names / codes	Environment Water – aquatic photolysis (major)
M47	BYI 02960-azabicyclosuccinamid		Environment Western Austic photolygic
	H	$C_{12}H_{14}F_2N_2O_4$	Environment
	0 H H	288.25 g/mol	Water – agratic photolysis
			(major)
	N H	BCS-CS64875	
	N CO <sub>2</sub> H	(Tested as BYI 02960-	
		azabiovologuacin wide Na	
	F´ `F	Salt, BCS-CU93236)	
M48	BYI 02960-succinamide	C <sub>12</sub> H <sub>13</sub> ClF <sub>2</sub> N <sub>3</sub> O <sub>3</sub>	Environment Water – amatic photolysis (major)  Environment Water – Aquatic photolysis (major)
11110	0	C <sub>12</sub> H <sub>13</sub> ClF <sub>2</sub> O <sub>3</sub>   C <sub>12</sub>	Favironment
		306 60 algori	Water Xquati Photo Qcic
	N CO <sub>2</sub> H	300.09 g/sp01	(mais A are priority sis
	F	DCS 671770 4	(major)
		DC9-0K/4/20	
<u> </u>	BYI 02960-succinamide  CI N F CO <sub>2</sub> H		Egyvironment Water – Aquatic photowsis (major)
	<u>~</u>		
	-Q,"		
	<i>O</i> 1 5		
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
	<b>V</b> '		
	& Ö <sup>Y</sup>		
			<i>7.</i> • • • • • • • • • • • • • • • • • • •
			• • • • • • • • • • • • • • • • • • •
		U ~ .?	
			J.
			- -
			U I
	Q' 4' Ä		
		U' 419 U &	
		C <sub>12</sub> H <sub>13</sub> ClF <sub>2</sub> N <sub>3</sub> O <sub>3</sub> 306.69 g/0001  BCS-OR747209  4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	
, S			
Ą			
\$*************************************			
\$ 5 m			
Ą	BYI 02960-succinamide  CI N F CO <sub>2</sub> H  CI N F F		
Ą			
Ļ			
Ą			
Å,			Water — Aquatic photowsis (major)
Å,			
		C <sub>12</sub> H <sub>13</sub> CIF <sub>3</sub> N <sub>3</sub> O <sub>3</sub> 306.69 g/mol  BCS-OR74729  January Color of the color of t	