Document Title

Tier 2 Summary of the Ecotoxicological studies on the Plant Protection Product for Sivanto (Flupyradifurone, BYI 02960) SL200

Spec. number 102000021884

Data Requirements

Regulation (EC) No 1107/2009

Annex IIIA
Section 6, Point 10
Document M

According to OECD format guidance for industry data submissions on plant protection products and their active substances

Date

2012-04-20

Author(s)

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Bayer CropScience

OWNERSHIP STATEMENT

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IIIA1 10 Ecotoxicological studies on the plant protection product

The formulation BYI 02960 SL200 is the representative formulation for the registration of the active substance Flupyradifurone (BYI 02960) in Europe. The following are addressed as safe-uses for the registration. The ecotoxicological risk assessment for these uses is included in this document.

A list of metabolites addressed in this section is included at the end, a full list is compiled in Document N.

Table 10- 1 Intended use pattern

Crop	F or G*	Timing of application	Number of applications	Application interval [days]	Maximum label rate [L/ha]	Maximum application rate, individual treatment [g/ha] BYI 02960
Hops	F	31 - 75	1	-	0.750	150
Lettuce [1)]	F	12 - 49	1	-	0.625	125
Lettuce [1)]	G	12 - 49	2	10	0.625	125

*F Field use G Glasshouse use

[1)] Head and leafy lettuce

Formulation density according to Section 1, point 2.6.1: 1.174 g/cm^3

The use in glasshouses is not specifically addressed in the document as the exposure of non-target organisms would be expected to be lower than from field uses, currently there are no EU agreed models to assess the exposure from glasshouses, and therefore this use is assumed to be covered by the field uses even considering the use of two applications in the glasshouse.

IIIA1 10.1 Effects on birds

Toxicity of the active substance to birds

The summary of the toxicity profile of the active substance BYI 02960 to birds is provided in the following Table 10.1- 1. Details of the studies concerned are provided in the Tier II summary documents on the active substances Annex II, Point 8.1.

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Table 10.1- 1: Avian toxicity data of BYI 02960

Test species	Test design	Ecotoxicological endpoint	Reference
Bobwhite Quail *(Colinus virginianus)*	acute, oral	LD_{50} 232 mg a.i./kg bw	[redacted] & [redacted] (2010) M-386036-01-1 KIIA 8.1.1/01
Canary *(Serinus canaria)*	acute, oral	LD_{50} 330 mg a.i./kg bw	[redacted] & [redacted] (2011) M-408514-01-1 KIIA 8.1.1/02
Chicken *(Gallus gallus domesticus)*	acute, oral	LD_{50} > 2000 mg a.i./kg bw	[redacted] & [redacted] (2011), M-420519-01-2 KIIA 8.1.1/03
Geometric mean		$LD_{50, geomean}$ 535 mg a.i./kg bw	
Endpoint for Tier 1 acute risk assessment		**LD_{50} 232 mg a.i./kg bw**	
Mallard Duck (*Anas platyrhynchos*)	5-day-feeding	LC_{50} > 4741 mg a.i./kg diet ≡ > 825 mg a.i./kg bw/d NOAEL 2238 mg a.i./kg diet ≡ 459 mg a.i./kg bw/d	[redacted] *et al.* (2010) M-388718-01-1 KIIA 8.1.2/01
Bobwhite Quail *(Colinus virginianus)*	5-day-feeding	LC_{50} > 4876 mg a.i./kg diet ≡ > 470 mg a.i./kg bw/d NOAEL 1133 mg a.i./kg diet ≡ 170 mg a.i./kg bw/d	[redacted] & [redacted] (2010) M-394535-01-1 KIIA 8.1.2/02
Mallard Duck (*Anas platyrhynchos*)	20-week feeding chronic, reproduction	NOAEL ≥ 845 mg a.i./kg diet ≡ ≥ 81 mg a.i./kg bw/d	[redacted] *et al.* (2011) M-412917-02-1 KIIA 8.1.4/01
Bobwhite Quail *(Colinus virginianus)*	23-week feeding chronic, reproduction	NOAEL 302 mg a.i./kg diet ≡ 40 mg a.i./kg bw/d	[redacted] *et al.* (2012) M-424704-01-1 KIIA 8.1.4/02
Endpoint for Tier 1 reproductive risk assessment		232 / 10 = **23.2 mg a.i./kg bw/d**	Lowest acute LD_{50} divided by 10

Bold letters: Values considered relevant for Tier 1 risk assessment

Metabolites of BYI 02960

The residues of BYI02960 have been investigated after application on cereals (as surrogate of undergrowth in hop yards) and on lettuce, at stages of potential relevance as food for herbivorous birds. The major component of the residue was the parent and only traces quantities of metabolites. Therefore, metabolites were not considered separately for risk assessment on birds

Toxicity of the formulated product

The acute oral toxicity of the formulated product was determined in studies on Bobwhite quail (Colinus virginianus) and in Chicken (*Gallus gallus domesticus*). There is no indication that the formulation is more toxic than expected based on the active substance content.

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Table 10.1- 2: Avian toxicity data of the formulated BYI 02960 SL 200 G

Test species	Test design	Ecotoxicological endpoint			Reference
Chicken *(Gallus gallus domesticus)*	acute, oral	LD_{50}	> 2000	mg prod./kg bw	[redacted] & [redacted] (2012) M-423043-01-2, KIIIA1 10.1.6/02
Northern Bobwhite Quail (*Colinus virginianus*)	acute, oral	LD_{50}	431	mg a.i./kg bw	[redacted] & [redacted] (2012) M-424312-01-1 KIIIA1 10.1.6/01

For more details reference is made to Point 10.1.6 of this dossier.

Selection of endpoints for the risk assessment
According to the Guidance Document on Risk Assessment for Birds & Mammals, EFSA 2009[1]

Acute risk assessment: where acute tests for more than one species are available the geometric mean may be used for the refined assessment, except when the endpoint for the most sensitive species is more than a factor 10 below the geometric mean of all the tested species. For BYI 02960 only Tier 1 acute risk assessments have been performed therefore the lowest LD_{50} of 232 mg/kg bw is used.

Short-term risk assessment; According to the risk assessment scheme a short-term risk assessment is not required. However, the endpoint from short-term dietary studies, e.g. 5-day dietary study in birds (OECD 205) should be used in an acute risk assessment when indicating a higher toxicity via the dietary exposure rout (lower LDD_{50}). In the case of BYI 02960 there is no indication that 5-day exposure via dietary route would provoke higher toxicity than one application via gavage in acute study and therefore the short-term toxicity is not considered in the acute risk assessment.

Reproductive risk assessment: The acute oral LD_{50} value used in the acute avian assessment should be divided by 10 for comparison with the lowest NOAED from the reproduction study (studies) ignoring purely parental effects (e.g. changes in parental body weight and food consumption). The lower of the values should be used in the tier 1 risk assessment.

For BYI 02960 the endpoint from the reproduction study (40 mg/kg bw/d) is not lower than the 1/10th of the LD_{50} = 232 mg/kg bw that has been proposed for use in the Tier 1 acute risk assessment.

Therefore, the endpoint of 23.2 mg/kg bw (1/10th of the lowest LD_{50} in Table 10.1- 1) will be used in a highly conservative Tier 1 reproductive risk assessment for birds.

Risk assessment for birds
The risk assessment procedure follows the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009).

At Tier 1, the risk is considered acceptable, if the 'Toxicity Exposure Ratio' (TER) value pass the trigger values of ≥ 10 for acute exposure and ≥ 5 for chronic exposure.

If the TER values are below these a-priori acceptability trigger values in certain areas, a refined risk assessment based on more relevant and realistic conditions is performed for those particular areas.

[1] EFSA (2009): Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. The EFSA Journal (2009), 7(12):1438.

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Calculation of Toxicity Exposure Ratio (TER)

According to the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009), the calculation of acute and long-term Toxicity to Exposure Ratio (TER) is defined as follows:

Acute risk: $TER_A = LD_{50}$ [mg as/kg bw] / DDD_{AC}

Long-term risk: $TER_{LT} = LD_{50}/10$ [mg as/kg bw] / DDD_{mean}

Calculation of Daily Dietary Dose (DDD)

Acute exposure DDD_{AC}:

The daily dietary dose for a single application per crop is given by the following equation:

DDD_{ACac} = application rate [kg/ha] × shortcut value (SV_{90})

Long-term exposure DDD_{mean}:

For a single application the daily dietary dose is given by the following equation:

DDD_{mean} = application rate [kg/ha] × shortcut value (SV_m) × f_{TWA}

Where

DDD	Daily dietary dose
f_{TWA}	Time weighted average factor f_{twa} based on a default time window of 21 days and a DT_{50} of 10 days leading to a value of 0.53
90	90th percentile values for acute exposure
m	mean values for reproductive/long-term exposure

Standard exposure scenario for Tier 1 risk assessment

The main potential exposure route for birds is expected to be consumption of contaminated feed.

Accordingly this will be main part of the risk assessment in the following under Sections 10.1.1 and 10.1.2.

The Tier 1 risk assessment is based on generic focal species associated with specific crop scenarios.

Default ("shortcut"-) values for the exposure estimate will be used as provided in Appendix A of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009) representing a worst case assessment.

In the Tier 1 risk assessment it is assumed that

- animals satisfy their entire food demand in the treated area (PT = 1),
- over an acute time frame (hours) the animals feed on items containing maximum residues (90th percentile), whereas they would ingest food containing mean residues over a long-term period (days to weeks),
- long-term predicted environmental concentrations to be compared with chronic endpoints can be calculated as the time-weighted average concentration. Default assumptions are a time window of 21 days and a DT_{50} of 10 days leading to a time weighted average factor (= f_{twa}) of 0.53. This factor is equally valid for feed items consisting of vegetation and for arthropods.

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Avian generic focal species for Tier 1 risk assessment

The product is intended to be used in hops at BBCH 31-75 and in lettuce at BBCH 12-49.

According to the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009) the following generic focal species are addressed in Tier 1 risk assessment.

Table 10.1- 3: Relevant generic avian focal species for Tier 1 risk assessment

Crop	Growth stage (BBCH)	Generic focal species	Representative species	Shortcut value	
				For long-term RA based on RUD_m	For acute RA based on RUD_{90}
Hops	≥ 20	Small insectivorous bird "finch"	Chaffinch	**10.6**	**25.3**
	20-39	Small granivorous bird "finch"	Goldfinch	**5.7**	**12.3**
	≥ 40	Small granivorous bird "finch"	Goldfinch	3.4	7.4
Lettuce (Leafy vegetables)	10-19	Medium herbivorous/granivorous bird "pigeon"	Wood pigeon	**37.0**	**90.6**
	10-49	Small granivorous bird "finch"	Serin	**12.6**	**27.4**
	10-49	Small omnivorous bird "lark"	Woodlark	**10.9**	**24.0**
	10-19	Small insectivorous bird "wagtail"	Yellow wagtail	**11.3**	**26.8**
	≥ 20	Small insectivorous bird "wagtail"	Yellow wagtail	9.7	25.2

Bold values were used for Tier 1 risk assessment. Where the same focal species is representative for different BBCH stages, only the worst-case SV values were chosen for the Tier 1 risk assessment.

Summary of calculated TER values for birds

Table 10.1- 4: Summary of all acute TER calculations as given under point 10.1.1

Crop (BBCH)	Generic focal species	Active substance	SV_{90}	TER_A	Assessment step
Hops (≥ 20)	Small insectivorous bird "finch" (Chaffinch)	BYI 02960	25.3	61	Tier 1
Hops (20-39)	Small granivorous bird "finch" (Goldfinch)		12.3	126	Tier 1
Lettuce (10-19)	Medium herbivorous/granivorous bird "pigeon" (Wood pigeon)	BYI 02960	90.6	20	Tier 1
Lettuce (10-49)	Small granivorous bird "finch" (Serin)		27.4	68	Tier 1
Lettuce (10-49)	Small omnivorous bird "lark" (Woodlark)		24.0	77	Tier 1
Lettuce (10-19)	Small insectivorous bird "wagtail" (Yellow wagtail)		26.8	69	Tier 1

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Table 10.1- 5: Summary of all reproductive (long-term) TER calculations as given under point 10.1.2

Crop (BBCH)	Generic focal species	Active substance	SV_m	TER_{LT}	Assessment step
Hops (≥ 20)	Small insectivorous bird "finch" (Chaffinch)	BYI 02960	10.6	28	Tier 1
Hops (20-39)	Small granivorous bird "finch" (Goldfinch)		5.7	51	Tier 1
Lettuce (10-19)	Medium herbivorous/granivorous bird "pigeon" (Wood pigeon)	BYI 02960	37.0	9	Tier 1
Lettuce (10-49)	Small granivorous bird "finch" (Serin)		12.6	28	Tier 1
Lettuce (10-49)	Small omnivorous bird "lark" (Woodlark)		10.9	32	Tier 1
Lettuce (10-19)	Small insectivorous bird "wagtail" (Yellow wagtail)		11.3	31	Tier 1

Conclusion: According to the presented conservative Tier 1 risk assessment that was based on the lowest LD_{50} the risk to birds from the use of the product in hops and lettuce is acceptable.

IIIA1 10.1.1 Acute toxicity exposure ratio (TER_A) for birds

Tier 1 acute toxicity exposure ratio for birds

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

Crop (BBCH)	Generic focalspecies	LD_{50} [mg/kg bw]	DDD			DDD	TER_A	Trigger
			Appl. rate [kg/ha]	SV_{90}	MAF_{90}			
BYI 02960								
Hops (≥ 20)	Small insectivorous bird "finch"	232	0.150	25.3	1	3.80	61	10
Hops (20-39)	Small granivorous bird "finch"			12.3		1.85	126	
Lettuce (10-19)	Medium herbiv./ granivorous bird "pigeon"	232	0.125	90.6	1	11.33	20	10
Lettuce (10-49)	Small granivorous bird "finch"			27.4		3.43	68	
Lettuce (10-49)	Small omnivorous bird "lark"			24.0		3.0	77	
Lettuce (10-19)	Small insectivorous bird "wagtail"			26.8		3.35	69	

The TER values for both crops are above the trigger of 10 for acute exposure. Hence, no unacceptable acute risk to birds from the use of the product according to the intended use pattern is to be expected.

Acute risk assessment for birds drinking contaminated water

An assessment of the risk potentially posed by consumption of contaminated drinking water is required according to the EFSA Guidance Document for Birds and Mammals (2009). For details see point 10.1.2 of this dossier.

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When the product is applied in hops, the formation of pools in leaf axils where an acute exposure possibly might occur can be excluded.

Acute risk assessment for the leaf scenario (use in lettuce):

The exposure of birds to drinking water from pools in leaf whorls as outlined above cannot be fully excluded from the use of the product in lettuce.

The respective calculations for birds have to be performed only for acute exposure. Generic focal species is a small granivorous bird (body weight 15.3 g) with a DWR (daily drinking water rate) of 0.46 L/kg bw/d. It should be mentioned that DWR, unlike FIR in the case of food intake exposure assessment, is already related to body weight.

According to EFSA Guidance Document for Birds and Mammals (2009), the PEC_{pool} calculates as follows:

$PEC_{pool} = C_{spray} / 5$ [mg/L]

$C_{spray} = 0.025$ kg/hL = 25 g/hL = 0.25g/L = 250 mg/L

The PEC_{pool} (= PEC_{dw}) is 250 / 5 = 50 mg/L for BYI 02960. The calculation of DDD (here: daily drinking water uptake) and TER are as follows:

$DDD = DWR * PEC_{dw}$

$TER_A = LD_{50}$ [mg as/kg bw/d] / DDD [mg as/kg bw/d]

Table 10.1.2- 4: Tier 1 acute DDD and TER calculation for exposure via drinking water from pools in leaf axils or on leaves

Compound	DWR [L/kg bw/d]	PEC_{dw} [mg/L]	DDD [mg/kg bw/d]	LD_{50} [mg/kg bw]	TER_A	Trigger
BYI 02960	0.46	50	23	232	10.1	10

The TER_{AC} value for drinking water exposure exceeds the a-priori acceptability trigger of 10 in a worst case risk assessment (highest concentration in spray water volume, lowest LD_{50}). Hence, no unacceptable risk is to be expected from the use of the product according to the intended use pattern.

The acute risk from water in puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil is covered by the long-term risk assessment under Point 10.1.2 of this dossier.

IIIA1 10.1.2 Short-term toxicity exposure ratio (TER_{ST}) for birds

Tier 1 short-term toxicity exposure ratio for birds

According to the risk assessment scheme of EFSA GD birds and mammals (2009) a short-term risk assessment is not required for BYI 02960.

Tier 1 long-term toxicity exposure ratio for birds

As described under the selection of end-points, the following are proposed for the long-term risk assessment for birds.

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Table 10.1.2- 1: Selection of endpoints for the use in long-term risk assessments for birds

BYI 02960	Lowest LD_{50} scenario	Geometric mean LD_{50} scenario
LD_{50}/10 (mg/kg bw)	23.2	53.5
NOAEL (mg/kg bw/d)	40	40
Endpoint for use in Tier 1 reproductive risk assessment	**23.2**	40

In the following conservative Tier 1 reproductive risk assessment, the lowest LD_{50}/10 = 23.2 mg/kg bw is employed as the long-term endpoint.

Table 10.1.2- 2: Tier 1 long-term DDD and TER calculation for birds

Crop (BBCH)	Generic focal species	NO(A)EL [mg/kg bw/d]	DDD				DDD	TER_{LT}	Trigger
			Appl. rate [kg/ha]	SV_m	MAF_m	f_{TWA}			
BYI02960									
Hops (≥ 20)	Small insectivorous bird "finch"	23.2	0.150	10.6	1.0	0.53	0.84	28	5
Hops (20-39)	Small granivorous bird "finch"			5.7			0.45	51	
Lettuce (10-19)	Medium herbiv./graniv. bird "pigeon"	23.2	0.125	37.0	1.0	0.53	2.45	9	5
Lettuce (10-49)	Small granivorous bird "finch"			12.6			0.83	28	
Lettuce (10-49)	Small omnivorous bird "lark"			10.9			0.72	32	
Lettuce (10-19)	Small insectivorous bird "wagtail"			11.3			0.75	31	

The TER values for both crops are above the trigger of 5 for reproductive/long-term exposure. Hence, no unacceptable risk is to be expected from the use of the product.

Long-term risk assessment for birds drinking contaminated water

An assessment of the risk potentially posed by consumption of contaminated drinking water is required according to the EPSA Guidance Document for Birds and Mammals (2009).

Due to the incidental nature of occurrence of drinking water reservoirs on agricultural fields (as compared to the contamination of food items growing or dwelling on those fields), a separate assessment of this exposure route is considered appropriate at least on the first-tier level.

Two scenarios were identified as relevant for assessing the risk of pesticides via drinking water to birds and mammals:

- Leaf scenario, only relevant for birds possibly drinking water from puddles in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation. This scenario is only relevant for acute exposure. As the product is intended to be applied in lettuce, the risk of birds drinking from puddles in leaf whorls cannot be excluded. Hence, a corresponding risk assessment has been performed in chapter IIIA 10.1.1.

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- Puddle scenario. Birds and mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for long-term exposure.

An "escape clause" recommended in the EFSA Guidance Document for Birds and Mammals (2009) allows for screening the need for a quantitative risk assessment by a comparison between the application rate and the toxicity of the respective substance. This escape clause specifies that *"due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals ..., no specific calculations of exposure and TER are necessary when the ratio of effective application rate* (= application rate x MAF) *(in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg)."* [2].

Table 10.1.2- 3: Evaluation of potential concern for exposure of birds drinking water (escape clause)

Compound	Koc [mL/g]	Application rate * MAF [g a.i./ha]	NO(A)EL [mg a.i./kg bw/d]	Ratio (Application rate * MAF) / NO(A)EL	"Escape clause" No concern if ratio	Conclusion
BYI 02960	98.4 [1]	150 [2] x 1	40	3.75	≤50	No concern

[1] Arithmetic mean of six K_{OC} values (see Section 5)

[2] Worst-case application rate in hops

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

IIIA1 10.1.3 In the case of bait, the concentration of active substance in the bait

Not applicable for spray application / seed treatment.

IIIA1 10.1.4 In the case of pellets, granules, prills or treated seed

Not applicable for spray application.

IIIA1 10.1.4.1 Amount of a.s. in or on each pellet, granule, prill or treated seed

Not applicable for spray application.

IIIA1 10.1.4.2 Proportion of the DT_{50} for the a.s. in 100 particles / gram particles

Not applicable for spray application.

IIIA1 10.1.5 In the case of pellets, granules and prills, their size and shape

Not applicable for spray application.

[2] EFSA (2009): Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA, p. 66

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IIIA1 10.1.6 Acute oral toxicity of the preparation to the more sensitive species

The acute oral toxicity of the formulated product was determined in a study on Bobwhite quail (*Colinus virginianus*) and, to address specific national requirement outside Europe, in a study on chicken (*Gallus gallus domesticus*). There is no indication that the formulation is more toxic than expected based on it its active substance content.

Report:	**KIIIA1 10.1.6/01; [redacted], T.L., [redacted], M.T (2012)**
Title:	Toxicity of BYI 02960 SL 200 During an Acute Oral LD_{50} with the Northern Bobwhite Quail (*Colinus virginianus*)
Report No:	EBRVP093
Document No:	M-424312-01-1
Guidelines:	**OPPTS 850.2100**
Deviations:	**None**
GLP:	**Yes (certified laboratory)**

Executive Summary

The aim of the study was to determine the acute effects of BYI 02960 SL 200 (Batch no. 2010-001067; Sample description: TOX 08907-00; Specification No.: 102000021884-01; Analysed content of a.i.: 17.1% w/w, 201.0 g/L) to northern bobwhite quail (*Colinus virginianus*).

Birds were orally dosed with BYI 02960 SL 200 based on body weight at dose levels of 250, 500, 1000, 2000, and 4000 mg a.i./kg body weight, respectively, using ten birds per dose level (five males and five females). Treatment levels were selected based on a descending geometric progression from the highest dose of 4000 mg a.i./kg body weight, and established to determine the LD_{50} value. Birds were dosed by oral gavage on Day 0 and were monitored for 21 days post-dosing. Adult body weights were taken on experimental Day -1, 7, 14, and 21, respectively. Feed consumption and clinical observations occurred daily. Post-mortem examinations were conducted on all remaining birds sacrificed at study termination and all birds found dead during the study. In addition, 10 control birds were kept under the same conditions from the beginning of the test until day 21, where all birds where sacrificed.

Mortality, signs of intoxication, food consumption, body weight and gross necropsy were used to determine the endpoints.

The acute oral LD_{50} for BYI 02960 SL 200 in northern bobwhite quail was 431 mg a.i./kg body weight (95% CL = 320 to 576 mg a.i./kg body weight). The lowest lethal dose was 250 mg a.i./kg body weight.

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MATERIAL AND METHODS

A. Materials

1. Test material

Test item:	BYI 02960 SL 200
Specification number:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Batch number:	2010-001067
Sample description:	TOX 08907-00
Nominal content of active ingredient:	BYI 02960 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.1% w/w, 201.0 g/L according to certificate of analysis
Density:	1.175 g/mL at 20 °C
Stability of test compound:	Expiry date 14.06.2012, when stored at 25 ± 5°C in original container in the dark (also acceptable from +2 to +30°C)
Application via…:	Oral gavage
Negative control:	Deionised water

2. Test organisms

Species:	*Colinus virginianus*
Common name:	Northern bobwhite quail
Age:	14 weeks
Source:	[redacted]
Feeding during test:	Teklad® Bayer Game Bird Ration
Body weight at test start:	Males: 180.8 to 196.3 g (range of both control and treatment group) Females: 176.1 to 192.8 (range of both control and treatment group)
Maintenance prior to test:	
Food:	Teklad® Bayer Game Bird Ration
Drinking water:	Tap water, *ad libitum*
Period of acclimation to test conditions:	14 days
Starvation period prior to test start:	15 hours
Mortality during acclimation period:	One

B. Study design and methods

1. In life dates August 02 to 23, 2011

2. Experimental treatments

Birds were housed by sex and dose level in stainless steel brooder type cages (L x B x H: 91 cm x 61cm x 25 cm), which were placed indoors. There were two cages per dose level, one containing the males and one containing the females. After 2 weeks of acclimation and 16 hours of starvation, 10 birds per dose level (5 males, 5 females group) were orally administered in a single oral dose by oral gavage using a syringe and a stainless steel animal feeding needle. Birds were dosed with BYI 02960 SL 200 based on body weight at dose levels of 250, 500, 1000, 2000, and 4000 mg a.i./kg bw. The intended treatment levels in protocol for the definitive study were designated as 25, 50, 100, 200, and 400 mg a.i./kg bw. However, due to a calculation error, the birds were actually dosed at 10x the intended treatment levels resulting in dose levels presented above. The higher dose levels caused total mortality at the dose levels of 1000, 2000, and 4000 mg a.i./kg body weight, but provided an adequate

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dose-response in the lower treatments of 500 and 250 mg a.i./kg body weight. Therefore, the treatment levels used for the study provided an adequate dose-response, calculation and slope of the LD_{50}. The deviation had no impact on the quality of the study. In addition, 10 control birds were kept under the same circumstances. Birds were monitored for 21 days post-dosing. Apart from the fasting period prior to dosing, all feed and water was provided *ad libitum*. Bodyweights were determined 1 day prior to dosing for the calculation of the individual test substance amounts.

3. Observation and measurements

Adult body weights were taken on experimental Day -1, Day 7, Day 14, and Day 21. Feed consumption and clinical observations occurred daily. The birds were observed twice daily (once on weekends and at study termination) during the treatment period for any mortalities and to detect any overt signs of toxicity or other clinical signs. The birds were observed three times on Day 0 following compound administration which occurred at approximately 1, 2, and 5 hours post-dosing.

Post-mortem examinations were conducted on all remaining birds sacrificed at study termination and all birds found dead during the study. At study termination surviving birds were sacrificed by CO2 asphyxiation. All surviving birds remaining in the study were necropsied due to the high levels of mortalities that occurred during the study.

4. Statistical Analysis

Mortality data were analyzed with CT-TOX a multi-method program that can determine the LC_{50} and 95% confidence interval using non-linear interpolation, Binomial, Moving Average, Probit, and Spearman-Karber methods.

Descriptive statistics (mean and standard deviation) for body weights and feed consumption were calculated in Microsoft® Excel. No statistical analysis was performed due to the high mortality rate in the 500, 1000, 2000, and 4000 mg a.i./kg body weight dose levels.

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RESULTS AND DISCUSSION

A. Environmental Conditions

Birds were kept under conditions which are summarised as follows:

Test temperature:	22°C (mean)
Relative humidity:	58% (mean)
Photoperiod:	8 hours light / 16 hours dark
Light intensity:	285 Lux
Air changes:	20 changes per hour

B. Biological Findings

Mortality and behavior:

The number of bird moralities during the study were: control (0), 250 (1), 500 (6), 1000 (10), 2000 (10), and 4000 (10) mg a.i./kg body weight, respectively. No clinical signs of toxicity or mortality occurred in the control group. All birds appeared normal immediately following dosing with no observed regurgitation. Observations of immobility were present for two birds in the 250 mg a.i./kg body weight group in which one bird died and the other recovered. In the 500 mg a.i./kg body weight group, four female birds were observed with signs of hypo-reactivity (lethargy) and/or immobility, of which three died and one recovered. All five of the male birds in the 500 mg a.i./kg body weight group were observed with signs of hypo-reactivity. Four of the male birds recovered with the fifth bird developing signs of immobility and was found dead on Day 3. All birds in the 1000, 2000 and 4000 mg a.i./kg body weight group were observed with signs of hyporeactivity and/or immobility, and died.

Pathological findings at necropsy:

Post-mortem examinations were generally unremarkable, with the exception that several birds were found with fluid in their crops.

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Table 10.1.6- 1: Acute oral toxicity of BYI 02960 SL 200 to northern bobwhite (*C. virginianus*): Post-mortem examination of northern bobwhite

Necropsy						
Treatment Level (mg ai/kg bw)	Control	250	500	1000	2000	4000
Total Bird Mortality	0	1	5	10	10	10
Number Birds Sacrificed	10	9	5	0	0	0
Number Birds Examined	10	10	10	10	10	10
General Symptoms						
Feather Loss	0	0	2	5	0	2
Skin Lesions/Abrasions	0	0	0	0	0	0
Emaciated	0	0	0	0	0	0
Liver & Heart						
Liver Lesions/Growths	0	0	0	0	0	0
Liver Enlarged	0	0	0	0	0	0
Heart Lesions/Growths	0	0	0	0	0	0
GI Tract						
Mouth Lesions/Growths	0	0	0	0	0	0
Esophagus Lesions/Growths	0	0	0	0	0	0
Proventriculus Lesions/Growths	0	0	0	0	0	0
Intestines Lesions/Growths	0	0	0	0	0	0
Crop Content:						
No food/grit	8	6	5	6	2	4
½ Full	2	1	1	1	1	1
Full	0	2	4	3	7	5
Proventriculus/Gizzard Content:						
No food/grit	1	1	0	1	1	2
½ Full	1	1	3	5	3	6
Full	8	8	7	4	6	2
Kidney & Spleen						
Kidney Lesions/Growths	0	0	0	0	0	0
Spleen Lesions/Growths	0	0	0	0	0	0
Postmortem Autolytic Signs						
Fluid filled Crop	0	0	4	5	10	10
Fluid filled Intestines	0	0	0	0	0	0
Gas filled Intestines	0	0	0	1	0	0

Table 10.1.6- 2: Acute oral toxicity of BYI 02960 SL 200 to northern bobwhite (*C. virginianus*): Mean body weight of male and female northern bobwhite quail

Male Body Weight								
Treatment Level (mg ai/kg bw)	Male Body Weight (g)							
	Day 0		Day 7		Day 14		Day 21	
	Mean ± S.D.	*n*	Mean ± S.D.	*n*	Mean ± S.D.	*n*	Mean ± S.D.	*n*
Control	189.6 ± 7.0	5	195.1 ± 10.6	5	197.3 ± 10.9	5	197.8 ± 9.9	5
250	188.7 ± 5.9	5	192.5 ± 3.5	4	195.2 ± 3.6	4	197.4 ± 4.3	4
500	188.4 ± 6.8	5	176.3 ± 20.4	4	187.1 ± 10.5	4	198.5 ± 7.7	4
1000	188.9 ± 6.3	5	–	0	–	0	–	0
2000	189.8 ± 6.0	5	–	0	–	0	–	0
4000	189.2 ± 6.9	5	–	0	–	0	–	0
Female Body Weight								
Treatment Level (mg ai/kg bw)	Female Body Weight (g)							
	Day 0		Day 7		Day 14		Day 21	
	Mean ± S.D.	*n*	Mean ± S.D.	*n*	Mean ± S.D.	*n*	Mean ± S.D.	*n*
Control	185.1 ± 5.6	5	191.4 ± 6.3	5	195.6 ± 6.5	5	196.3 ± 6.3	5
250	184.8 ± 5.5	5	185.8 ± 8.6	5	191.6 ± 9.0	5	194.5 ± 9.6	5
500[a]	184.2 ± 6.0	5	159.6	1	196.5	1	184.7	1
1000	184.9 ± 6.0	5	–	0	–	0	–	0
2000	184.5 ± 4.5	5	–	0	–	0	–	0
4000	184.6 ± 5.4	5	–	0	–	0	–	0

[a]Value reported when only 1 surviving bird present

Table 10.1.6- 3: Acute oral toxicity of BYI 02960 SL 200 to northern bobwhite (*C. virginianus*): Mean body weight changes of male northern bobwhite quail

Male Body Weight Change						
Treatment Level (mg ai/kg bw)	Male Body Weight Change (g)					
	Day 0 to 7		Day 7 to 14		Day 0 to 14	
	Mean ± S.D.	*n*	Mean ± S.D.	*n*	Mean ± S.D.	*n*
Control	5.5 ± 6.2	5	2.2 ± 1.2	5	7.7 ± 6.0	5
250	4.9 ± 3.3	4	2.7 ± 1.0	4	7.6 ± 3.4	4
500	-13.2 ± 14.7	4	20.8 ± 10.4	4	7.6 ± 6.4	4
1000	–	0	–	0	–	0
2000	–	0	–	0	–	0
4000	–	0	–	0	–	0

Male Body Weight Change						
Treatment Level (mg ai/kg bw)	Male Body Weight Change (g)					
	Day 0 to 21		Day 7 to 21		Day 14 to 21	
	Mean ± S.D.	*n*	Mean ± S.D.	*n*	Mean ± S.D.	*n*
Control	8.2 ± 5.3	5	2.0 ± 2.2	5	0.5 ± 1.6	5
250	9.8 ± 2.9	4	4.9 ± 1.9	4	2.2 ± 0.8	4
500	9.0 ± 2.0	4	22.2 ± 13.7	4	1.4 ± 3.7	4
1000	–	0	–	0	–	0
2000	–	0	–	0	–	0
4000	–	0	–	0	–	0

Table 10.1.6- 4: Acute oral toxicity of BYI 02960 SL 200 to northern bobwhite (C. virginianus): Mean body weight changes of female northern bobwhite quail

Female Body Weight Change						
Treatment Level (mg ai/kg bw)	Female Body Weight Change (g)					
	Day 0 to 7		Day 7 to 14		Day 0 to 14	
	Mean ± S.D.	*n*	Mean ± S.D.	*n*	Mean ± S.D.	*n*
Control	5.9 ± 3.4	5	4.6 ± 0.5	5	10.5 ± 3.7	5
250	0.6 ± 4.0	5	6.1 ± 2.2	5	6.8 ± 5.3	5
500[a]	-16.5	1	16.9	1	0.4	1
1000	–	0	–	0	–	0
2000	–	0	–	0	–	0
4000	–	0	–	0	–	0

Female Body Weight Change						
Treatment Level (mg ai/kg bw)	Female Body Weight Change (g)					
	Day 0 to 21		Day 7 to 21		Day 14 to 21	
	Mean ± S.D.	*n*	Mean ± S.D.	*n*	Mean ± S.D.	*n*
Control	11.2 ± 2.8	5	5.3 ± 1.[illegible]	[illegible]	0.7 ± 1.4	[illegible]
250	9.7 ± 6.8	5	9.1 ± 3.9	5	3.0 ± 1.9	5
500[a]	8.6	1	6.1	1	8.2	1
1000	–	0	–	0	–	0
2000	–	0	–	0	–	0
4000	–	0	–	0	–	0

[a]Value reported when only 1 surviving bird present.

Table 10.1.6- 5: Acute oral toxicity of BYI 02960 SL 200 to northern bobwhite (*C. virginianus*): Mean daily feed consumption of male and female northern bobwhite quail

Mean Daily Feed Consumption (g/bird/day)		
Treatment Level (mg ai/kg bw)	Female 21-day Exposure (Mean ± Standard Deviation)	Male 21-day Exposure (Mean ± Standard Deviation)
Control	19.3 ± 5.8	20.8 ± 9.3
250	31.3 ± 7.8	24.3 ± 9.4
500	28.5[a]	16.6 ± 5.5
1000	–	–
2000	–	–
4000	–	–

[a]Value reported when only 1 surviving bird present.

D. Biological Endpoints Derived

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

LD_{50}:	431 mg a.i./kg body weight
Lowest lethal dose (LLD):	250 mg a.i./kg b.w.

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CONCLUSION

The acute oral LD_{50} for BYI 02960 SL 200 in northern bobwhite quail was 431 mg a.i./kg b.w. (95% CL = 320 to 576 mg a.i./kg body weight). The lowest lethal dose was 250 mg a.i./kg b.w.

Report:	**KIIIA1 10.1.6/02; [redacted], [redacted] (2012)**
Title:	Acute oral toxicity of chicken (*Gallus gallus domesticus*) with BYI 2960 SL200, according to OECD 223 - Limit test -
Report No:	BAR/LD 142
Document No:	M-423043-01-2
Guidelines:	**OECD Guideline 223**
Deviations:	**The observation period was prolonged to 21 days in order to be in compliance with some national requirements.**
GLP:	**Yes (certified laboratory)**

Executive Summary

The aim of the study was to determine the acute effects of BYI 02960 SL 200 (Batch ID: 2011-002192; Sample description: TOX 09373-00; Master recipe ID: 0113814-001; Specification No.: 102000021884-02; Analysed content of a.i.: 17.1% w/w, 201.4 g/L) to hens (Gallus gallus domesticus) in a limit test.

Five adult hens (treatment group) were observed during a period of 21 days after 2000 mg form./kg body weight had been administered orally via gelatine capsule. In addition, 10 control birds were kept under the same conditions from the beginning of the test until day 21, where 5 birds where sacrificed.

Mortality, signs of intoxication, food consumption, body weight and gross necropsy were used to determine the endpoints.

The acute oral LD50 was determined to be > 2000 mg form./kg body weight, the non-lethal dose was found to be ≥ 2000 mg form./kg body weight.

MATERIAL AND METHODS

A. Materials

1. Test material

Test item:	BYI 02960 SL 200
Specification number:	102000021884-02
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Batch number:	2011-002192
Material number:	79718845
Sample description:	TOX 09373-00
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.1% w/w, 201.4 g/L
Density:	1.175 g/mL at 20 °C
Stability of test compound:	Expiry date: 04.04.2013, when stored at 25 ± 5°C in original container in the dark (also acceptable from +2 to +30°C)
Application via…:	Gelatine capsule
Negative control:	Deionised water

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2. Test organisms

Species:	*Gallus gallus domesticus*
Common name:	Hen
Age:	18 weeks
Source:	[redacted]
Feeding during test:	Standard rearing diet for hens ([redacted]
Body weight at test start:	1170 to 1460 g (range of both control and treatment group)
Maintenance prior to test:	
Food:	Standard rearing diet for hens ([redacted]
Drinking water:	Tap water, *ad libitum*
Period of acclimation to test conditions:	14 days
Starvation period prior to test start:	16 hours
Mortality during acclimation period:	None

B. Study design and methods

1. In life dates September 13 to October 18, 2011

2. Experimental treatments

After 2 weeks of acclimation and 16 hours of starvation, 5 birds (treatment group) were orally administered with gelatine capsules containing 2000 mg form./kg b.w. as limit. 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. Dosing was followed by a subsequent observation period of 28 days. Bodyweights were determined 1 day prior to dosing for the calculation of the individual test substance amounts.

Birds were housed individually, in stainless steel wire racks (L x B x H: 88 cm x 33 cm x 43 cm), which were placed indoors.

3. Observation and measurements

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

Body weights were recorded prior to test initiation (day -1), on study day 3, 7, 14 and 21 (test termination).

Food consumption was measured daily until day 3, then for the periods 3-7, 7-14 and 14-21. On study days 1, 2, 3, 7 and 14 all remaining food was replaced by fresh food after cleaning. At the end of the study all surviving birds were sacrificed by CO_2 asphyxiation. Gross necropsies were carried out on all survivors at the end of the study.

RESULTS AND DISCUSSION

A. Environmental Conditions

Birds were kept under conditions which are summarised as follows:

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Test temperature:	21.2 °C (mean)
Relative humidity:	55.6% (mean)
Photoperiod:	14 hours light / 10 hours dark
Light intensity:	Not stated
Air changes:	Not stated

B. Biological Findings

Mortality and behaviour:
No mortality occurred in both control and treatment.

After the application the dosed birds avoided food almost completely. 4 of 5 birds started to feed from day +3 on, the last one from day +7 on. Afterwards they behave as the control birds. The treated birds showed different signs of impairment on the day of application (e.g. tremor, reduced vigilance or apathy). On the following days mainly impacts on the digestive tract were observed like excretion of uric acid, diarrhea or soft experiment which disappeared from day +8 on. Afterwards, 2 of the treated birds showed slight signs of digestive stress as soft excrements on single days, but on the majority of days and at test termination all birds were free of any symptoms.

Body weight development:
The body weight development was not impacted.

Pathological findings at necropsy:
No pathological findings at the necropsy of control and dosed birds.

Table 10.1.6- 6: Summarized signs of intoxication

Cage No	Dose Level (mg form./kg b.w.)	Observed Effects
1	Control	OB
2	Control	OB
3	Control	OB
4	Control	OB
5	Control	OB
6	Control	OB
7	Control	OB
8	Control	OB
9	Control	OB
10	Control	OB
11	2000	DM,S,RV,PT,TR,UA,SE
12	2000	S,SE,UA
13	2000	S,RV,DM,DR,UA,SE
14	2000	S,RV,DM,UA,DR,SE
15	2000	S,RV,DM,AP,TR,UA,DR, SE

OB = no symptoms found; S = sitting; RV = reduced vigilance; TR = tremor;
DM = discoordinated movements; DR = diarrhea; UA = uric acid; SE = soft excrements;
AP = apathy; PT = ptosis

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Table 10.1.6- 7: Food consumption, hen/day (g)

	Food consumption hen/day (g)					
	day 0	**day 1**	**day 2**	**day 3-7**	**day 7-14**	**day 14-21**
Cage 1 / Control	104	101	89	85	88	84
Cage 2 / Control	96	79	76	77	60	68
Cage 3 / Control	93	62	60	62	68	69
Cage 4 / Control	139	128	93	96	95	107
Cage 6 / Control	89	74	75	65	75	84
Cage 6 / Control	128	91	84	87	100	99
Cage 7 / Control	124	88	90	80	98	96
Cage 8 / Control	103	81	81	85	89	89
Cage 9 / Control	82	82	73	73	75	80
Cage 10 / Control	112	93	82	83	87	94
Mean:	**107**	**88**	**80**	**79**	**83**	**87**
Cage 11 / 2000 mg form./kg b.w.	7	0	10	66	107	100
Cage 12 / 2000 mg form./kg b.w.	7	1	2	17	75	94
Cage 13 / 2000 mg form./kg b.w.	4	2	1	69	84	96
Cage 14 / 2000 mg form./kg b.w.	17	2	27	86	79	86
Cage 15 / 2000 mg form./kg b.w.	2	10	17	97	79	96

C. Validity Criteria

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

D. Biological Endpoints Derived

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

LD_{50}: > 2000 mg form./kg body weight
Non-lethal dose (NLD): ≥ 2000 mg form./kg b.w.

CONCLUSION

The acute LD_{50} for chicken dosed (oral) with BYI 02960 SL 200 in a limit test was > 2000 mg form./kg b.w. The non-lethal dose was ≥ 2000 mg form./kg b.w.

IIIA1 10.1.7 Supervised cage or field trials

The risk assessment based on the active substances indicates acceptable acute, short-term and long-term risks to birds (see Points 10.1.1 and 10.1.2 of this dossier). For this reason and also considering animal welfare, no supervised cage or field study with the preparation was deemed necessary.

IIIA1 10.1.8 Acceptance of bait, granules or treated seed by birds

Not applicable for spray application.

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IIIA1 10.1.9 Effects of secondary poisoning

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log P_{OW} > 3 is used to trigger an in-depth evaluation of the potential for bioaccumulation. Summaries of the Log P_{ow} studies are given in Section 1, Point 2 for the active substance and Section 5, point 7.13 for the metabolites).

Table 10.1.9- 1: Log Pow values of BYI 02960 and its metabolites

Substance	log P_{ow}	Reference
BYI 02960	1.2 (pH 7, 25°C)	[redacted] & [redacted] (2011), M-414485-01-1
BYI 02960-succinamide	-1.3 (pH 7, 23°C)	[redacted] & [redacted] (2011), M-416883-01-1
BYI 02960-azabicyclosuccinamide	-2.7 (pH 7, 23°C)	[redacted] & [redacted] (2011), M-416656-01-1
DFA	-3.1 (pH 7, 23°C)	[redacted] & [redacted] (2011), M-416624-01-1
BYI 02960-DFEAF	-0.2 (pH 7, 22°C)	[redacted] et al. (2011) , M-414259-01-1
6-CNA	1.52 (25°C)	[redacted] (2001), M-204285-01-1

As neither the active ingredient nor any of its metabolites has a log P_{ow} above 3, the potential of bioaccumulation is considered to be very low. Hence, there is no need to evaluate potential effects of secondary poisoning of birds.

IIIA1 10.2 Effect on aquatic organisms

Toxicity of BYI 02960 to aquatic organisms

A summary of the aquatic toxicity profile for BYI 02960 and its metabolites is provided in Table 10.2-1 and Table 10.2- 2). Details of the studies concerned are provided in the Tier II summary document on the active substance Annex II, Point 8.2 to 8.6 and 8.11.

Table 10.2- 1: Toxicity of BYI 02960 to aquatic organisms

Test species	Test system	Test duration	Endpoint [mg a.i. /L]	Reference
BYI 02960-fish				
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	LC_{50} > 74.2 (mm)[2] NOEC ≥ 74.2 (mm)	[redacted] & [redacted] (2010) M-390611-01-1 KIIA 8.2.1.1/01
Pimephales promelas (fathead minnow)	static acute	96 h	LC_{50} > 70.5 (mm) NOEC ≥ 70.5 (mm)	[redacted] & [redacted] (2010) M-392560-01-1 KIIA 8.2.1.2/01
Cyprinus carpio (carp)	static acute	96 h	LC_{50} > 100 (mm) NOEC ≥ 100 (mm)	[redacted] (2011) M-420407-01-2 KIIA 8.2.1.2/02
Pimephales promelas (fathead minnow)	early life stage (ELS), flow-through	35 d	NOEC 4.41 (mm) LOEC 8.41 (mm)	[redacted] & [redacted] (2011) M-409339-01-1 KIIA 8.2.4/01
BYI 02960-invertebrates				
Daphnia magna (water flea)	static acute	48 h	EC_{50} > 77.6 (mm) NOEC ≥ 77.6 (mm)	[redacted] & [redacted] (2009) M-357476-01-1 KIIA 8.3.1.1/01
Daphnia magna (water flea)	chronic, static renewal	21 d	NOEC 3.2 (nom)[3] LOEC 6.4 (nom)	[redacted] (2011) M-414066-01-2 KIIA 8.3.2.1/01
Chironomus riparius (chironomid)	static acute	48 h	EC_{50} 0.062 (nom) NOEC 0.025 (nom)	[redacted] (2011) M-414739-01-2 KIIA 8.3.1.2/01
Chironomus riparius (chironomid)	Static chronic, spiked water	28 d	NOEC 0.0105 (mi) EC_{50} 0.0353 (mi)	[redacted] (2011) M-401792-01-2 KIIA 8.3.2.2/01
BYI 02960- algae and plants				
Pseudokirchneriella subcapitata (green alga)	growth inhibition static	96 h	E_rC_{50} > 80 (nom) NOE_rC ≥ 80 (nom)	[redacted] & [redacted] (2010) M-397552-01-1 KIIA 8.4/01
Lemna gibba (duck weed)	growth inhibition static renewal	7 d	$E_bC_{50\ (frond\ no.)}$ > 67.7 (mm) $E_rC_{50(frond\ no)}$ > 67.7(mm)	[redacted], *et al.* (2010) M-398376-01-1 KIIA 8.6/01
BYI 02960- Marine organisms				
Cyprinodon variegatus (sheepshead minnow)	static acute	96 h	LC_{50} > 83.9 (mm) NOEC 83.9 (mm)	[redacted] & [redacted] (2009) M-357479-01-1 KIIA 8.11.1/01
Crassostrea virginica (eastern oyster)	acute, flow-through	96 h	EC_{50} > 29 (mm) NOEC ≥ 29 (mm)	[redacted], *et al.*(2009) M-361668-01-1 KIIA 8.11.1/02
Americamysis bahia (saltwater mysid)	flow-through	96 h	EC_{50} 0.26 (mm) NOEC 0.12 (mm)	[redacted], *et al.*(2009) M-364620-01-1 KIIA 8.11.1/03
Americamysis bahia (saltwater mysid)	Life cycle, flow-through	28d	NOEC 0.0132 (mm) LOEC 0.0236 (mm)	[redacted], *et al* (2011) M-420783-01-1 KIIA 8.11.1/04
BYI 02960- Amphibians				
Xenopus laevis (African clawed frog)	Static acute	48 h	LC_{50} > 73.8 (mm) NOEC ≥ 73.8 (mm)	[redacted] & [redacted] (2011) M-417822-01-1 KIIA 8.2.1.1/02

[2] mm = mean measured concentration, [3] nom = nominal concentration, [4] mi = initially measured concentration

.

Bold values: Endpoints considered relevant for risk assessment

Table 10.2- 2: Toxicity of BYI 02960 metabolites to aquatic organisms

Test species	Test system	Test duration	Endpoint [mg p.m.[1]/L]	Reference
BYI 02960 – succinamide				
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	**LC_{50} > 100 (nom)** NOEC ≥ 100 (nom)	[redacted] (2011) M-414293-01-1 KIIA 8.2.1.3/02
Daphnia magna (water flea)	chronic, static renewal	21 d	**NOEC 43.3 (nom)** LOEC 100 (nom)	[redacted] (2012) M-424700-01-2 KIIA 8.3.2.1/02
Chironomus riparius (chironomid)	static acute	48 h	**EC_{50} > 100 (mi)** [4] NOEC 71 (mi)	[redacted] (2011) M-417386-01-2 KIIA 8.3.1.2/02
Pseudokirchneriella subcapitata (green alga)	growth inhibition test	72 h	E_rC_{50} > 10 (nom) NOE_rC ≥ 10 (nom)	[redacted] (2011) M-414090-01-2 KIIA 8.4/03
BYI 02960 – azabicyclosuccinamide				
Chironomus riparius (chironomid)	static acute	48 h	**EC_{50} > 100 (mi)** [4] NOEC 71 (mi)	[redacted] (2011) M-424404-01-1 KIIA 8.3.1.2/03
DFA (tested as Sodium difluoro acetate)				
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	**LC_{50} > 10 (nom)** NOEC ≥10 (nom)	[redacted] (2011) M-413889-01-1 KIIA 8.2.1.3/02
Daphnia magna (water flea)	static acute	48 h	**EC_{50} > 10 (nom)** NOEC 10 (nom)	[redacted] (2011) M-409326-01-1 KIIA 8.3.1.1/02
Chironomus riparius (chironomid)	static chronic, spiked water	28 d	LOEC > 100 (nom) **NOEC ≥ 100 (nom)**	[redacted] (2011) M-415913-01-2 KIIA 8.3.2.2/02
Pseudokirchneriella subcapitata (green alga)	growth inhibition static	72 h	**E_rC_{50} > 10 (nom)** NOE_rC 10 (nom)	[redacted] (2011) M-409118-01-2 KIIA 8.4/02
6-Chloronicotinic acid				
Daphnia magna	acute, static renewal	48 h	**EC_{50} > 95.1 (mm)** NOEC 95.1 (mm)	[redacted] (1997) M-196569-01-1 KIIA 8.3.1.1/03
Chironomus tenants (chironomid)	static acute	96 h	**LC_{50} 1 (mi)** [4] NOEC 1 (mi)	[redacted] and [redacted] (1998) M-048448-01-1 KIIA 8.3.1.2/04
Chironomus riparius (chironomid)	static chronic, spiked water	28 d	LOEC > 100 (nom) **NOEC ≥ 100 (nom)**	[redacted] (2011) M-416604-01-2 KIIA 8.3.2.2/03
Pseudokirchneriella subcapitata **(green alga)**	growth inhibition test	72 h	**E_rC_{50} > 100 [A] (nom)** NOEC ≥ 100 (nom)	[redacted] (2012) M-424145-01-2 KIIA 8.4/04

[1] p.m. = pure metabolite in case of studies on metabolites, [2] mm = mean measured concentration
[3] nom = nominal concentration, [4] mi = initially measured concentration
[A] E_rC_{50} at a test concentration of 100 mg pure metabolite/L, pH adjusted (pH 7.5 - 8.2)

Bold values: Endpoints considered relevant for risk assessment

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Toxicity of the formulated product

A summary of the aquatic toxicity profile of BYI 02960 SL 200 G is provided in Table 10.2- 3.

Table 10.2- 3: Toxicity of BYI 02960 SL 200 G to aquatic organisms

Test species	Test system	Test duration	Endpoint [mg a.i./L]	Reference
Freshwater organisms				
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	LC_{50} >105 (mm)[1] NOEC <105(mm)	[redacted] (2011) M-398721-02-2 KIIIA1 10.2.2.1/01
Cyprinus carpio (common carp)	static acute	96 h	LC_{50} 108 (mm) NOEC 108 (mm)	[redacted] (2011) M-420485-01-2 KIIIA1 10.2.2.1/02
Daphnia magna (water flea)	static acute	48 h	EC_{50} 684 mg form./L (equivalent to 117.0 (mm)) NOEC 125 mg form./L (equivalent to21.4 (mm))	[redacted] (2010) M-393738-01-2 KIIIA1 10.2.2.2/01
Chironomus riparius (chironomid)	chronic test – spiked water	28 d	**NOEC 0.012 (mi)** [2] LOEC 0.024 (mi) $EC_{15\ emergence\ rate}$ 0.0132 (mi) $EC_{15\ developmental\ rate}$ 0.0233 (mi)	[redacted] (2011) M-416145-01-2 KIIIA1 10.2.6.2/01
Pseudokirchneriella subcapitata (green alga)	growth inhibition test	72 h	E_rC_{50} > 250 mg form./L (equivalent to 42.5 (mm)) NOEC > 250 mg form./L (equivalent to 42.5 (mm))	[redacted] (2010) M-397244-01-2 KIIIA1 10.2.2.3/01

[1] mm = mean measured concentration
[2] mi = initially measured concentration
Bold value: Endpoint considered relevant for risk assessment

Overview on aquatic effects data for BYI 02960

The active ingredient of BYI 02960 SL200 G, BYI 02960 has been tested on all laboratory indicator species for aquatic organisms representing all trophic levels of the aquatic environment. Acute data on fish toxicity are available for four different species, amongst them the sensitive cold water fish trout, and the salt water fish, Cyprinodon variegatus. The results indicate that BYI 02960 is practically non-toxic to fish. As such, the LC50 values reported are greater than the highest concentration within the test. Using the formulated product BYI 02960 SL 200G the LC50 as related to the active substance was 105 mg a.i./L.

An early life-stage test for BYI 02960 confirms the low toxicity to fish also on chronic scale. Exposed continuously over the full developmental life-phase of fathead minnow a slightly reduced fry survival at the highest test concentration of 10 mg a.i./L was recorded. As compared to the untreated control survival was reduced by 7% resulting in an overall fry survival of 87.5% at a mean measured concentration of 8.4 mg a.i./L. Time to hatch and hatching success was not influenced, neither was any effect observed on growth as determined on length and weight.

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A very selective non-target activity of BYI 02960 is further confirmed by low toxicity to representatives of three other taxonomic groups, namely amphibians, algae and aquatic plants. There were no adverse effects noted in the 48h acute frog test, and the influence of the active substance on Pseudokirchneriella subcapitata and Lemna gibba was also negligible. In a test with the formulation no effects on algal development were recorded at the highest test concentration used 250 mg formulation/L corresponding to 42.5 mg a.i./L. For Lemna, an onset of effects was determined at a mean measured concentration of 67.7 mg a.i./L , aquatic plants are also considered to be insensitive to the active substance.

BYI 02960 is an insecticide against sucking insects. Hence, a side activity against aquatic invertebrates can be anticipated. Data were generated on all laboratory standard indicator species of aquatic arthropods: the cladoceran *Daphnia magna*, the midge *Chironomus riparius* and, the marine species *Americamysis bahia* and the mollusc *Crassostrea virginica*. The most sensitive organism for BYI 02960 was found to be *Chironomus riparius*. While acute toxicity to daphnids was negligible with a NOEC for the active substance at or greater than 77.6 mg a.i./L, the 50% effect concentration (EC50) to the midge was determined to be at a concentration of 0.062 mg a.i./L. For the saltwater species, the LC50 of the mysid shrimp was at 0.26 mg a.i./L and indicates as such a lower sensitivity than the insect. BYI 02960 was also non-toxic to the marine oyster.

Chronic tests confirm the high toxicity of the active substance to *Chironomus riparius*. The lowest-observed-effect concentration (LOEC) impacting the emergence of the developed Chironomus larvae was at 0.02 mg a.i./L, only a factor of 3 below the acute EC50 values. The acute-to-chronic ratio for the most sensitive ecotoxicological endpoint, survival of Chironomus, for BYI 02960 is thus low. The immediate activity observed in the acute test appears to be reflected in the chronic test.

The 28 day chronic mysid shrimp test resulted in a LOEC of 0.0236 mg a.i./L based upon a statistically significant influence on reproductive output. From the first-tier laboratory test species indicative for a potential effect of an insecticide Chironomus is considered to be of higher relevance for the risk assessment than the marine shrimp as it reflects a real taxonomic group present in the freshwater biocoenosis. Hence, it is considered to supersede the data available for the mysid shrimp. The influence of the formulation BYI 02960 SL 200 G was therefore examined on Chironomus as the most sensitive invertebrate. The lowest-observed-effect concentration was at 0.024 mg a.i./L, the NOEC was established at 0.012 mg a.i./L. There was no relevant influence of the formulation on the toxicity in comparison to the toxicity with the active substance.

Hence, the first-tier risk assessment will use endpoints from the formulation and the active substance. In case of the representative standard laboratory species Chironomus riparius the NOEC of 12 µg a.i./L (formulation test) and the LOEC of 21.3 µg a.i./L (lowest LOEC, observed in the active substance test) needs to be taken into consideration for the risk assessment. For the acute first-tier risk assessment, the EC50 of 62 µg a.i./L is the relevant endpoint for aquatic invertebrates.

Metabolites of BYI 02960

Occurrence of metabolites from BYI 02960 in surface waters may have different origins following use of BYI 02960 SL 200 G. Direct entry of the active substance is anticipated from spray drift of the formulated product during application and indirect exposure of surface waters may also occur via drainage or run-off from the treated farmland. As there is currently no EU agreed procedure for assessing exposure following applications in glasshouses this use is assumed to be covered by the field use in lettuce.

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BYI 02960 is degraded in soil to two major metabolites, DFA and 6-CNA which may therefore potentially enter surface water bodies following formation in soil, additionally DFA was formed in aerobic water/sediment studies. In aquatic systems, under the influence of photolysis two major degradates BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide were formed, these may occur in water following entry of BYI 02960. The testing strategy for the metabolites was discussed in the Annex II, Point 6 and it was concluded that the metabolites are not of ecotoxicological relevance.

IIIA1 10.2.1 Toxicity exposure ratios for aquatic species

A detailed description of the predicted concentrations in surface water is provided in Annex III, section 5, Point 9.7 and 9.8.

Concentrations in groundwater were also considered, as groundwater might become surface water, leading to exposure of aquatic organisms. The PECgw (see Annex III, Section 5 point 9.6.1 and 9.6.2) values for the active substance and the metabolite 6-CNA are lower than the PECsw values for all FOCUS scenarios and application rates (for details see Point 9.6) and thus, not relevant for the risk assessment. However for the metabolite DFA with maximum Tier 1 groundwater PEC values higher than the PECsw (Table 10.2.1-3). Hence, the risk assessment for this metabolite will be conducted with the PECgw. In the risk assessment further dilution of the groundwater was not considered, therefore this tier 1 risk assessment provides a very conservative approach.

The relevant PEC values considered for TER calculations are summarised in the tables below. The calculation with Step 1 values was omitted.

Table 10.2.1- 1: Maximum PECsw values of BYI 02960 and its metabolites following application of BYI 02960 SL 200 G to hops (steps 2 to 3)

		Hops (1 x 150 g a.i./ha) $PEC_{sw, max}$ [µg/L]				
FOCUS Scenario	Buffer [m]	**BYI 02960**	**-succinamide**	**-azabicyclo-succinamide**	**DFA**	**6-CNA**
STEP 2						
Northern EU	--	13.07	4.065	2.499	0.743	0.232
Southern EU	--	**17.36**	**4.065**	**2.499**	1.268*	**0.463**
STEP 3						
R1 (pond)	--	**0.394**	-	-		-
R1 (stream)	--	**5.531**	-	-		-

Bold values were used for risk assessments
* Groundwater predictions for DFA exceed surface water values and are therefore used in the risk assessment (see Table 10.2.1-3)

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Table 10.2.1- 2: Maximum PECsw values of BYI 02960 following application of BYI 02960 SL 200 G to hops (step 4)

		Hops (1 x 150 g a.i./ha) $PEC_{sw, max}$ [µg/L]			
STEP 4 (incl. drift reduction)		**BYI 02960**			
Drift reduction		**0%**	**50%**	**75%**	**90%**
	Buffer (m)				
R1 (stream)	-	5.531	2.766	1.383	**0.553**
R1 (stream)	5	4.515	2.258	**1.129**	0.452
R1 (stream)	10	2.354	1.177	0.589	0.235
R1 (stream)	20	0.708	0.354	0.177	0.071

Bold values were used for risk assessments

Table 10.2.1- 3: Maximum PECsw values of BYI 02960 and metabolites following application of BYI 02960 SL 200 G lettuce (step 2-3)

		Lettuce (1 x 125 g a.i./ha) $PEC_{sw, max}$ [µg/L]				
FOCUS Scenario	Buffer [m]	**BYI 02960**	**-succinamide**	**-azabicyclo-succinamide**	**DFA -**	**6-CNA**
STEP 2						
Northern EU	--	6.410	0.484	0.297	0.682	0.289
Southern EU	--	**11.78**	**0.484**	**0.297**	1.339*	**0.579**
STEP 3						
D3 (ditch, 1st)	--	**0.830**	-	-		-
D3 (ditch, 2nd)	--	**0.840**	-	-	-	-
D4 (pond, 1st)	--	**1.035**	-	-	-	-
D4 (stream, 1st)	--	**0.794**	-	-	-	-
D6 (ditch, 1st)	--	**1.268**	-	-	-	-
R1 (pond, 1st)	--	**0.060**	-	-	-	-
R1 (stream, 1st)	--	**0.858**	-	-	-	-
R1 (pond, 2nd)	--	**0.097**	-	-	-	-
R1 (stream, 2nd)	--	**1.186**	-	-	-	-
R2 (stream, 1st)	--	**1.586**	-	-	-	-
R2 (stream, 2nd)	--	**0.940**	-	-	-	-
R3 (stream, 1st)	--	**2.226**	-	-	-	-
R3 (stream, 2nd)	--	**3.570**	-	-	-	-
R4 (stream, 1st)	--	**0.522**	-	-	-	-
R4 (stream, 2nd)	--	**4.808**	-	-	-	-

Bold values were used for risk assessments

* Groundwater predictions (see Table 10.2.1-5) exceed surface water values for the metabolite Difluoroacetic acid (DFA) and are therefore used in the risk assessment;

Table 10.2.1- 4: Maximum PECsw values of BYI 02960 following application of BYI 02960 SL 200 G lettuce (step 4)

FOCUS Scenario	Lettuce (1 x 125 g a.i./ha) $PEC_{sw, max}$ [µg/L]			
STEP 4	**BYI 02960**			
Mitigation	50% DRN	5 m drift buffer	10 m drift and VFS	20 m drift and VFS
D3 (ditch, 1st)	**0.434**	**0.252**	**0.151**	0.097
D3 (ditch, 2nd)	**0.446**	**0.265**	**0.165**	0.110
D4 (pond, 1st)	1.034	1.035	**1.034**	**1.033**
D4 (stream, 1st)	0.721	0.721	**0.721**	**0.721**
D6 (ditch, 1st)	1.268	1.268	**1.268**	**1.268**
R1 (pond, 1st)	0.050	0.037	0.029	0.016
R1 (stream, 1st)	0.858	0.858	**0.389**	0.204
R1 (pond, 2nd)	0.087	0.094	0.043	0.024
R1 (stream, 2nd)	1.186	1.186	**0.540**	0.283
R2 (stream, 1st)	1.586	1.586	**0.716**	**0.375**
R2 (stream, 2nd)	0.940	0.940	**0.422**	0.220
R3 (stream, 1st)	2.226	2.226	**1.009**	**0.528**
R3 (stream, 2nd)	3.570	3.570	**1.630**	**0.856**
R4 (stream, 1st)	0.261	0.191	0.101	0.053
R4 (stream, 2nd)	4.808	4.808	**2.184**	**1.144**

Bold values were used for risk assessments

* Groundwater predictions (see Table 10.2.1-3 below) exceed surface water values for the metabolite Difluoroacetic acid (DFA) and are therefore used in the risk assessment;

VFS = vegetated filter strip DRN = Drift Reducing Nozzles

Table 10.2.1- 5: Maximum Tier 1 groundwater PEC values for the metabolite Difluoroacetic acid (DFA) in hops and lettuce

	Difluoroacetic acid (DFA) (PEARL), every year PEC_{gw} [µg/L]	
Scenario	Hops 1 x 150 g/ha of parent	Lettuce 1 x 125 g/ha of parent
Hamburg	**1.423**	**2.382**

Bold values were used for risk assessments

Risk assessment

The risk assessment is based on Guidance Document on Aquatic Ecotoxicology, SANCO/3268/2001, rev 4 final, 17 October 2002. Toxicity exposure ratios (TER values) are calculated based on the most sensitive species and worst-case PEC_{SW} values.

The TER-values have been calculated based on the following equations:

TER_A = LC_{50} or EC_{50} / maximum PEC_{SW}

TER_{LT} = E_rC_{50} / maximum PEC_{SW}

TER_{LT} = chronic NOEC /max or long-term PEC_{SW}

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The risk is considered acceptable if the TER_A values are ≥ 100, and the TER_{LT} values ≥ 10.

IIIA1 10.2.1.1 TER_A for fish

Table 10.2.1.1- 1: TER_A calculations for fish based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops						
BYI 02960	*P. promelas*	LC_{50}	> 70 500	17.36	> 4 061	100
BYI 02960 – succinamide	*O. mykiss*	LC_{50}	> 100 000	4.005	> 24 600	
DFA*	*O. mykiss*	LC_{50}	> 10 000	1.423	> 7 027	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

Table 10.2.1.1- 2: TER_A calculations for fish based on FOCUS Step 2 for application in lettuce

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Lettuce						
BYI 02960	*P. promelas*	LC_{50}	> 70 500	11.78	> 5 985	100
BYI 02960 – succinamide	*O. mykiss*	LC_{50}	> 100 000	0.484	> 206 612	
DFA*	*O. mykiss*	LC_{50}	> 10 000	2.382	> 4 198	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

The TER_A values meet the required trigger for both uses, indicating an acceptable acute risk to fish for application of the product.

IIIA1 10.2.1.2 TER_{LT} for fish

Table 10.2.1.2- 1: TER_{LT} calculations for fish based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Hops						
BYI 02960	*P. promelas*	NOEC	4410	17.36	254	10

Table 10.2.1.2- 2: TER_{LT} calculations for fish based on FOCUS Step 2 for application in lettuce

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*P. promelas*	NOEC	4410	11.78	374	10

The TER_{LT} values meet the required trigger for both uses, indicating an acceptable long-term risk to fish for application of the product.

IIIA1 10.2.1.3 TER_A for Daphnia

Table 10.2.1.3- 1: TER_A calculations for Daphnia based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops						
BYI 02960	*D. magna*	EC_{50}	> 77 600	17.36	> 4 470	100
DFA*	*D. magna*	EC_{50}	> 10 000	1.423	> 7 027	
6-CNA	*D. magna*	EC_{50}	> 95 100	0.463	> 205 400	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

Table 10.2.1.3- 2: TER_A calculations for Daphnia based on FOCUS Step 2 for application in lettuce

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Lettuce						
BYI 02960	*D. magna*	EC_{50}	> 77 600	11.78	> 6 587	100
DFA*	*D. magna*	EC_{50}	> 10 000	2.382	> 4 198	
6-CNA	*D. magna*	EC_{50}	> 95 100	0.579	> 164 249	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

The TER_A values meet the required trigger for both uses, indicating an acceptable acute risk to *Daphnia* for application of the product.

IIIA1 10.2.1.4 TER_{LT} for Daphnia

Table 10.2.1.4- 1: TER_{LT} calculations for Daphnia based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Hops						
BYI 02960	*D. magna*	NOEC	3 200	17.36	184	10
BYI 02960 – succinamide	*D. magna*	NOEC	43 300	4.065	10 652	

Table 10.2.1.4- 2: TER_{LT} calculations for Daphnia based on FOCUS Step 2 for application in lettuce

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*D. magna*	NOEC	3 200	11.78	272	10
BYI 02960 – succinamide	*D. magna*	NOEC	43 300	0.484	89 463	

The TER_{LT} values meet the required trigger for both uses, indicating an acceptable long-term risk to *Daphnia* for application of the product.

IIIA1 10.2.1.5 TER_A for an aquatic insect species

Table 10.2.1.5- 1: TER_A calculations for *C. riparius* based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops						
BYI 02960	*C. riparius*	EC_{50}	62	17.36	**4**	100
BYI 02960 –– succinamide	*C. riparius*	EC_{50}	> 100 000	4.065	> 24 600	
BYI 02960 - azabicyclosuccinamide	*C. riparius*	EC_{50}	> 100 000	2.499	> 40 016	
6-CNA	*C. tentans*	LC_{50}	1000	0.463	2 160	

Bold values do not meet the trigger

Table 10.2.1.5- 2: TER_A calculations for *C. riparius* based on FOCUS Step 2 for application in lettuce

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Lettuce						
BYI 02960	*C. riparius*	EC_{50}	62	11.78	**5**	100
BYI 02960 – succinamide	*C. riparius*	EC_{50}	> 100 000	0.484	> 206 612	
BYI 02960 – azabicyclosuccinamide	*C. riparius*	EC_{50}	> 100 000	0.297	336 700	
6-CNA	*C. tentans*	LC_{50}	1000	0.579	1 727	

Bold values do not meet the trigger

For BYI 02960 metabolites the TER meets the trigger at Step 2, for BYI 02960 the trigger is not met at Step 2 and therefore calculation considering the more realistic FOCUS Step 3 have been performed:.

Table 10.2.1.5- 3: TER_A calculations for *C. riparius* based on FOCUS Step 3 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Hops							
BYI 02960	*C. riparius*	EC_{50}	62	0.394	R1, pond	157	100
				5.531	R1, stream	**11**	

Bold values do not meet the trigger

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Table 10.2.1.5- 4: TER$_A$ calculations for *C. riparius* based on FOCUS Step 3 for application in lettuce

Compound	Species	Endpoint [µg/L]		PEC$_{sw,max}$ [µg/L]	Scenario	TER$_A$	Trigger
Crop: Lettuce							
BYI 02960	*C. riparius*	EC$_{50}$	62	0.830	D3 (ditch, 1st)	**75**	100
				0.840	D3 (ditch, 2nd)	**74**	
				1.035	D4 (pond, 1st)	**60**	
				0.794	D4 (stream, 1st)	**78**	
				1.268	D6 (ditch, 1st)	**49**	
				0.060	R1 (pond, 1st)	1033	
				0.858	R1 (stream, 1st)	**72**	
				0.097	R1 (pond, 2nd)	639	
				1.186	R1 (stream, 2nd)	**52**	
				1.586	R2 (stream, 1st)	**39**	
				0.940	R2 (stream, 2nd)	**66**	
				2.226	R3 (stream, 1st)	**28**	
				3.570	R3 (stream, 2nd)	**17**	
				0.522	R4 (stream, 1st)	119	
				4.808	R4 (stream, 2nd)	**13**	

Bold values do not meet the trigger

For use in hops and lettuce a safe use is shown at Step 3 for at least one scenario (TER > 100), potential mitigation measures are investigated considering Step 4 calculations.

Table 10.2.1.5- 5: Refined TER$_A$ calculations for *C. riparius* based on FOCUS Step 4 for application in hops (90 % drift reduction)

Compound	Species	Endpoint [µg/L]		PEC$_{sw,max}$ [µg/L]	Scenario	TER$_A$	Trigger
Crop: Hops; 90 % drift reduction (no buffer zone)							
BYI 02960	*C. riparius*	EC$_{50}$	62	0.553	R1, stream	112	100

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Table 10.2.1.5- 6: Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (drift reducing nozzles)

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Drift reduction [%]	Scenario	TER_A	Trigger
Crop: Lettuce; drift reduction (No buffer)							
BYI 02960	*C. riparius*	EC_{50} 62	0.434	50	D3 (ditch, 1st)	143	100
			0.446	50	D3 (ditch, 2nd)	139	
			1.033	90	D4 (pond, 1st)	**60**	
			0.721	90	D4 (stream, 1st)	**86**	
			1.268	90	D6 (ditch, 1st)	**49**	
			0.858	90	R1 (stream, 1st)	**72**	
			1.186	90	R1 (stream, 2nd)	**52**	
			1.586	90	R2 (stream, 1st)	**39**	
			0.940	90	R2 (stream, 2nd)	**66**	
			2.226	90	R3 (stream, 1st)	**28**	
			3.570	90	R3 (stream, 2nd)	**17**	
			4.808	90	R4 (stream, 2nd)	**13**	

Bold values do not meet the trigger

Table 10.2.1.5- 7: Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (5 m distance)

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Lettuce; 5 m drift buffer (no drift reduction)						
BYI 02960	*C. riparius*	EC_{50} 62	0.252	D3 (ditch, 1st)	246	100
			0.265	D3 (ditch, 2nd)	234	
			1.035	D4 (pond, 1st)	**60**	
			0.721	D4 (stream, 1st)	**86**	
			1.268	D6 (ditch, 1st)	**49**	
			0.858	R1 (stream, 1st)	**72**	
			1.186	R1 (stream, 2nd)	**52**	
			1.586	R2 (stream, 1st)	**39**	
			0.940	R2 (stream, 2nd)	**66**	
			2.226	R3 (stream, 1st)	**28**	
			3.570	R3 (stream, 2nd)	**17**	
			4.808	R4 (stream, 2nd)	**13**	

Bold values do not meet the trigger

Table 10.2.1.5- 8: Refined TER$_A$ calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (10 m distance)

Compound	Species	Endpoint [µg/L]		PEC$_{sw,max}$ [µg/L]	Buffer type	Scenario	TER$_A$	Trigger
Crop: Lettuce; 10 m distance (spray drift only (S) or VFS (R))								
BYI 02960	*C. riparius*	EC_{50}	62	1.034	S	D4 (pond, 1st)	**60**	
				0.721	S	D4 (stream, 1st)	**86**	
				1.268	S	D6 (ditch, 1st)	**49**	
				0.389	R	R1 (stream, 1st)	159	
				0.540	R	R1 (stream, 2nd)	115	100
				0.716	R	R2 (stream, 1st)	**87**	
				0.422	R	R2 (stream, 2nd)	147	
				1.009	R	R3 (stream, 1st)	**61**	
				1.630	R	R3 (stream, 2nd)	**38**	
				2.184	R	R4 (stream, 2nd)	**28**	

Bold values do not meet the trigger, VFS: vegetated filter strip

Table 10.2.1.5- 9: Refined TER$_A$ calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (20 m distance)

Compound	Species	Endpoint [µg/L]		PEC$_{sw,max}$ [µg/L]	Buffer type	Scenario	TER$_A$	Trigger
Crop: Lettuce; 20 m distance (spray drift only (S) or VFS (R))								
BYI 02960	*C. riparius*	EC_{50}	62	1.033	S	D4 (pond, 1st)	**60**	
				0.721	S	D4 (stream, 1st)	**86**	
				1.268	S	D6 (ditch, 1st)	**49**	
				0.375	R	R2 (stream, 1st)	165	100
				0.528	R	R3 (stream, 1st)	117	
				0.856	R	R3 (stream, 2nd)	**72**	
				1.144	R	R4 (stream, 2nd)	**54**	

Bold values do not meet the trigger, VFS, vegetated filter strip

Conclusion:

A safe use has been shown for the scenario R1 (pond) in both hops and lettuce with no risk mitigation. Additionally by applying mitigation measures an acceptable use can be shown for scenario D3 with no buffer zone and drift reducing nozzles, and for scenarios R1 stream, R2 stream and R3 stream considering different buffer zones (vegetated filter strips).

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IIIA1 10.2.1.6 TER_{LT} for an aquatic insect species

Table 10.2.1.6- 1: TER_{LT} calculations for *C. riparius* based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg a.i./L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Hops						
BYI 02960 SL 200	*C. riparius*	NOEC	12	17.36	**< 1**	10
DFA*	*C. riparius*	NOEC	≥ 100 000	1.423	≥ 70 274	
6-CNA	*C. riparius*	NOEC	≥ 100 000	0.463	≥ 215 983	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters, bold values do not meet the trigger

Bold values do not meet the trigger

Table 10.2.1.6- 2: TER_{LT} calculations for *C. riparius* based on FOCUS Step 2 for application in lettuce

Compound	Species	Endpoint [µg a.i./L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960 SL 200	*C. riparius*	NOEC	12	11.78	**1**	10
DFA*	*C. riparius*	NOEC	≥ 100 000	2.382	≥ 41 982	
6-CNA	*C. riparius*	NOEC	≥ 100 000	0.579	≥ 172 712	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters; bold values do not meet the trigger

Bold values do not meet the trigger

While the margin of safety for the metabolites DFA and 6- CNA is far above any level of concern, the TER_{LT} values for the parent compound do not meet the required trigger based on worst-case Step 2 PECsw values. Hence, further calculations based on more realistic **FOCUS Step 3 values** are required.

Table 10.2.1.6- 3: TER_{LT} calculations for *C. riparius* based on FOCUS Step 3 for application in hops

Compound	Species	Endpoint [µg a.i./L]		$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Hops							
BYI 02960 SL 200	*C. riparius*	NOEC	12	0.394	R1, pond	30	10
				5.531	R1, stream	**2**	

Bold values do not meet the trigger

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Table 10.2.1.6- 4: TER_{LT} calculations for *C. riparius* based on FOCUS Step 3 for application in lettuce

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960 SL 200	*C. riparius*	NOEC 12	0.830	D3 (ditch, 1st)	14	
			0.840	D3 (ditch, 2nd)	14	
			1.035	D4 (pond, 1st)	12	
			0.794	D4 (stream, 1st)	15	
			1.268	D6 (ditch, 1st)	**9**	
			0.060	R1 (pond, 1st)	200	
			0.858	R1 (stream, 1st)	14	
			0.097	R1 (pond, 2nd)	124	10
			1.186	R1 (stream, 2nd)	10	
			1.586	R2 (stream, 1st)	**8**	
			0.940	R2 (stream, 2nd)	13	
			2.226	R3 (stream, 1st)	**5**	
			3.570	R3 (stream, 2nd)	**3**	
			0.522	R4 (stream, 1st)	23	
			4.808	R4 (stream, 2nd)	**2**	

Bold values do not meet the trigger

The TER_{LT} values for aquatic insects do not meet the required trigger based upon Step 3 PECsw values in some scenarios. A refined risk assessment with **FOCUS Step 4 values,** considering mitigation options is required.

Table 10.2.1.6- 5: TER_{LT} calculations for *C. riparius* based on FOCUS Step 4 for application in hops with exposure mitigation measures

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Hops; no buffer zone 90% drift reduction						
BYI 02960 SL 200	*C. riparius*	NOEC 12	0.553	R1, stream	22	10
5 m distance (spray drift buffer) and 75 % drift reduction						
BYI 02960 SL 200	*C. riparius*	NOEC 12	1.13	R1, stream	11	10

Table 10.2.1.6- 6: TER_{LT} calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce with 10 m distance (VFS)

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Lettuce; 10 m drift and run-off buffer						
BYI 02960 SL 200	*C. riparius*	NOEC 12	1.268	D6 (ditch, 1st)	**9.5**	10
			0.716	R2 (stream, 1st)	17	
			1.009	R3 (stream, 1st)	12	
			1.630	R3 (stream, 2nd)	**7**	
			2.184	R4 (stream, 2nd)	**5**	

Bold values do not meet the trigger; VFS, vegetated filter strip

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Table 10.2.1.6- 7: TER_{LT} calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce with 20 m distance (VFS)

	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Lettuce 20 m drift and run-off buffer						
BYI 02960 SL 200	*C. riparius*	NOEC 12	1.268	D6 (ditch, 1st)	**9.5**	10
			0.856	R3 (stream, 2nd)	14	
			1.144	R4 (stream, 2nd)	10.5	

Bold values do not meet the trigger; VFS, vegetated filter strip

Conclusion:

Based on the calculations presented above a safe use of the formulation in hops and lettuce can be shown for all FOCUS scenarios except D6 considering different mitigation options (up to 20m VFS).

IIIA1 10.2.1.7 TER_A for an aquatic crustacean species

An acute toxicity test to a second crustacean species, *Americamysis bahia*, has been conducted with the active substance resulted in an EC50 of 0.26 mg/L (see Table 10.2-1). While the mysid shrimp represents a saltwater organism that is likely to differ in its response to freshwater crustacean as represented by Daphnia magna, the data show that the most sensitive organism is the insect Chironomus riparius. Thus, the risk assessment as based upon the midge will add an additional margin of safety as already established by the risk assessment for the other crustacean species, Daphnia magna.

IIIA1 10.2.1.8 TER_{LT} for an aquatic crustacean species

From the toxicity to *Daphnia magna* no particular concern for crustacean was indicated, instead the most sensitive organism identified was the insect *C. riparius*. Thus, the risk assessment as based upon the midge will add an additional margin of safety to crustacean species as already established for *Daphnia magna*.

IIIA1 10.2.1.9 TER_A for an aquatic gastropod mollusc species

No studies on aquatic gastropod molluscs are necessary since the product is not intended to be applied directly in/at surface water bodies. No hazard or risk is to be expected for these organisms as BYI 02960 showed a very selective activity against insects only. A marine species, the oyster *Crassostrea virginica* was tested acutely with the active substance and showed low sensitivity as compared to the aquatic insect *C. riparius*. Hence, the risk mitigation established for *Chironomus riparius* will add on the margin of safety already in place due to lower sensitivity of the mollusc.

IIIA1 10.2.1.10 TER_{LT} for an aquatic gastropod mollusc species

No studies on aquatic gastropod molluscs are necessary since the product is not intended to be applied directly in/at surface water bodies. No hazard or risk is to be expected for these organisms.

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IIIA1 10.2.1.11 TER_{LT} for algae

Table 10.2.1.11- 1: TER_{LT} calculations for algae based on FOCUS Step 2 following application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Hops						
BYI 02960	*P. subcapitata*	E_rC_{50}	> 80 000	17.36	> 4 608	10
BYI 02960 – succinamide	*P. subcapitata*	E_rC_{50}	> 10 000	4.065	> 2 460	
DFA*	*P. subcapitata*	E_rC_{50}	> 10 000	1.423	> 7027	
6-CNA	*P. subcapitata*	E_rC_{50}	> 100 000	0.463	> 215 983	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

Table 10.2.1.11- 2: TER_{LT} calculations for algae based on FOCUS Step 2 following application in lettuce

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*P. subcapitata*	E_rC_{50}	> 80 000	11.78	> 6 791	10
BYI 02960 – succinamide	*P. subcapitata*	E_rC_{50}	> 10 000	0.484	> 20 661	
DFA*	*P. subcapitata*	E_rC_{50}	> 10 000	2.382	> 4 198	
6-CNA	*P. subcapitata*	E_rC_{50}	> 100 000	0.579	> 172 712	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

The TER_{LT} values meet the required trigger for both uses considering Step 2 calculations, indicating an acceptable chronic risk to algae for application of the product.

TER_{LT} for higher aquatic plants

Table 10.2.1.11- 3: TER_{LT} calculations for aquatic plants based on FOCUS Step 2 following application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Hops						
BYI 02960	*L. gibba*	E_rC_{50}	> 67 700	17.36	> 3 900	10

Table 10.2.1.11- 4: TER_{LT} calculations for aquatic plants based on FOCUS Step 2 following application in lettuce

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*L. gibba*	E_rC_{50}	> 67 700	11.78	> 5 747	10

The TER_{LT} values for *Lemna* meet the required trigger for both uses at Step 2, indicating an acceptable chronic risk to higher aquatic plants for application of the product.

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IIIA1 10.2.2 Acute toxicity (aquatic) of the preparation

The formulated product BYI 02960 SL 200 G is intended for use as a spray formulation, therefore its toxicity was profiled by testing all relevant indicator species representative for key taxa of the aquatic ecosystem. Acute tests were performed on fish, daphnia and algae and, chronic toxicity was examined for the most sensitive species identified from testing with the active substance BYI 02960, the aquatic insect Chironomus riparius. The outcome of the tests showed no relevant influence of the formulation on the toxicity in comparison to the active substance.

IIIA1 10.2.2.1 Fish acute toxicity LC_{50}, freshwater, cold-water species

Report:	**KIIIA1 10.2.2.1/01; [redacted] E. (2011)**
Title:	Acute toxicity of BYI 02960 SL 200 G to fish (*Oncorhynchus mykiss*) under static conditions (limit test)
Report No:	EBRVP098
Document No:	M-398721-02-2
Guidelines:	**EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985); OPPTS 850.1075 (Public Draft, 1996); Council Regulation (EC) No 440/2008, C.1 (2008); OECD No. 203 (rev.1992)**
Deviations:	**None**
GLP	**Yes (certified laboratory)**

Executive Summary

A limit test at 100 mg a.i./L was performed in order to demonstrate that fish (Oncorhynchus mykiss) were not affected by BYI 02960 SL 200 G (Sample description: TOX 08907-00 (Batch ID: 2010-00106; Material No.: 79718845; Specification No.: 102000021884-01)) 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 a nominal concentration of 100 mg a.i./L against a water control with further 30 fish. Recoveries of BYI 02960 were measured in all test levels on day 0, day 2 and day 4 of the exposure period to confirm nominal concentrations.

Test conditions met all validity criteria, given by the guidelines.

The LC50 of BYI 02960 SL 200 G to Rainbow Trout (Oncorhynchus mykiss) in a static 96-hour-test was determined to be > 100 mg a.i./L.

In one of the test aquaria with 100 mg a.i./L one fish died. This is a mortality of 3% and is explainable by biological variability and does not influence the outcome of the study.

At this test level the fish showed the following symptoms after 96 hours: 29 showed labored respiration, 1 was dead.

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MATERIAL AND METHODS

A. Materials

1. Test material:

Test item:	BYI 02960 SL 200 G
Specification number:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Batch No.:	2010-001067
Material number:	79718845
Sample description:	TOX 08907-00
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.1% w/w, 201.0 g/L
Density:	1.175 g/ml at 20 °C
Stability of test compound:	Expiry date: 14.06.2012 when stored at 25 ± 3°C in original container in the dark (also acceptable from +2 to +30°C)

2. Vehicle and/or positive control:

Solvent control:	Untreated test water (negative control)
Positive control:	No positive control was tested

3. Test organisms:

Species:	*Oncorhynchus mykiss*
Common name:	Rainbow trout
Length:	Mean body total length 5.3 ± 0.9 cm (mean ± SD)
Body weight:	Mean body wet weight 1.7 ± 0.9 g (mean ± SD)
Source:	[redacted]
Acclimation period:	48 h (fish were observed for 14 days prior to testing)
Feed:	Commercial trout food ([redacted]) during acclimation period
Starvation:	Animals were not fed 48 h before and during the study
Biomass loading:	0.64 g fish/L test medium

B. Study design and methods

1. In life dates: 7 to 11 June 2010

2. Experimental treatments:

Based on a range finder test (non-GLP), the definitive test concentration was set at 100 mg test item/L (limit test). The test media with the different test concentrations were prepared by weighing an adequate amount of the test item and dissolving it in a part of the test water, before pouring it back into the test vessels.

The aquaria used were made of glass (size of 38 x 32 x 36 cm - h x w x d). The test volumes amounted to 40 L. For every test concentration two aquaria were used and were labeled with a study number, a number and the nominal concentration of the test item. The aquaria were placed in a temperature controlled room.

Immediately prior to the test, water samples were taken from the center of the aquaria for analytical determination of the active ingredient concentration. At the start of the test, thirty fish were randomly introduced.

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Thirty fish (fifteen fish per test vessel I and II) were exposed in a limit test for 96 h under static test conditions to a nominal concentration of 100 mg a.i./L against a water control (control I and II with 15 fish each).

3. Observation and measurements:

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

Within the study the pH-value, the oxygen saturation level and the temperature were measured with commercial measurement devices, daily.

Recoveries of BYI 02960 were measured in all test levels on day 0, day 2 and day 4 of the exposure period to confirm nominal concentrations.

RESULTS AND DISCUSSION

A. Environmental Parameters

Reconstituted water was used during the acclimation period and for the test. It was prepared by adding salt stock solutions to demineralized water. The test water was periodically analyzed for undesired impurities.

Photoperiod:	16 h light : 8 h darkness
Test water:	
Hardness:	40 – 60 mg $CaCO_3$/L
Dissolved oxygen (DO):	> 60% oxygen saturation
pH:	> 6.0 and < 8.0
Water temperature:	range of 10°C - 14°C
Conductivity:	< 0.2 mS/cm

B. Analytical Findings

Dissolved oxygen concentrations ranged from 78% to 97% oxygen saturation, the pH values ranged from 6.7 to 7.2 and the water temperature ranged from 11.0°C to 12.5°C in all aquaria over the whole testing period.

The analytical determination of BYI 02960 (in water by HPLC – MS / MS) revealed concentrations of 105% of nominal over the whole testing period of 96 hours at the limit test concentration of 100 mg a.i./L. Therefore, all results are given as nominal concentrations.

C. Biological Findings

There were neither any sub-lethal effects nor any mortality in the control group.

Cumulative mortality was observed as follows [with a total number of 30 fish at each concentration (15 I + 15 II)]:

Table 10.2.2.1- 1: Cumulative Mortality and Behavioral Observations

Exposure time	4 h		24 h		48 h		72 h		**96 h**	
Test item [mg a.i./L]	no. of dead	% dead	no. of dead	% dead	no. of dead	% dead	no. of dead	% dead	**no. of dead**	**% dead**
Control I	0	0	0	0	0	0	0	0	**0**	**0**
Control II	0	0	0	0	0	0	0	0	**0**	**0**
100 I	0	0	0	0	0	0	0	0	**0**	**0**
100 II	0	0	0	0	1	3	1	3	**1**	**3**

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D. Validity Criteria

Test conditions met all validity criteria, given by the mentioned guidelines. There was less than 5% mortality within the 48-hour settling-in period and ≤ 10% mortality in the controls. Dissolved oxygen saturation was greater or equal to 60% throughout the test and pH variation was ≤ 1.0 units.

E. Biological Endpoints Derived

The acute toxicity of BYI 02960 SL 200 to rainbow trout is summarized as followed:

Test item:	BYI 02960 SL 200 G
Test object:	Rainbow trout (*Oncorhynchus mykiss*)
Exposure:	96h, static , limit test
LC_{50} 96 h:	**> 100 mg a.i. / L**

CONCLUSION

In a limit test at measured concentration of 100 mg a.i./L of BYI 02960 SL 200 G in one aquarium one fish died. This is a mortality of 3% and is explainable by biological variability and does not influence the outcome of the study. No other mortality occurred in rainbow trout (*Oncorhynchus mykiss*), therefore the 96h-LC_{50} is above 100 mg a.i./L.

Report:	**KIIA1 10.2.2.1/02; [redacted] E. (2011)**
Title:	Acute toxicity of BYI 02960 SL 200 G to fish (*Cyprinus carpio*) under static conditions (limit test)
Report No:	EBRVP199
Document No:	M-420485-01-2
Guidelines:	**EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985); OPPTS 850.1075 (Public Draft, 1996); Council Regulation (EC) No 440/2008, C.1 (2008); OECD No. 203 (rev.1992); JMAFF, 12 Nousan No. 8147 (2000)**
Deviations:	**None**
GLP	**Yes (certified laboratory)**

Executive Summary

A limit test at 585 (100) mg test item (a.i.)/L was performed in order to demonstrate that fish (*Cyprinus carpio*) were not affected by BYI 02960 SL 200 G (Sample description: TOX 08907-00 (Batch ID: 2010-00106; Material No.: 79718845; Specification No.: 102000021884)) 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 a nominal concentration of 100 mg a.i./L against a water control and a solvent control with further 30 fish. Recoveries of BYI 02960 were measured in all test levels on day 0, day 2 and day 4 of the exposure period to confirm nominal concentrations.

Test conditions met all validity criteria, given by the mentioned guidelines. There was less than 5% mortality within the 48-hour settling-in period and ≤ 10% mortality in the controls. Dissolved oxygen saturation was greater or equal to 60% throughout the test and pH variation was ≤ 1.0 units.

The LC_{50} (96h) of BYI 02960 SL 200 G to Common carp *(Cyprinus carpio)* in a static 96-hour-test was determined to be > 585 (100) mg test item (a.i.)/L.

The NOLEC and the NOEC was at or greater than 585 (100) mg test item (a.i.)/L.

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MATERIAL AND METHODS

A. Materials

1. Test material:

Test item:	BYI 02960 SL 200 G
Specification number:	102000021884
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Batch No.:	2010-001067
Material number:	79718845
Sample description:	TOX 08907-00
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.1% w/w, 201.0 g/L
Density:	1.175 g/ml at 20 °C
Stability of test compound:	Expiry date: 14.06.2012 when stored at 25 ± 2°C in original container in the dark (also acceptable from +2 to +30°C)

2. Vehicle and/or positive control:

Solvent control:	Water control (negative control)
Positive control:	Reference substance: Copper (II) sulphate ($CuSO_4$) tested in a separate study: 96h-LC_{50} of 41.0 µg/L (App. H)

3. Test organisms:

Species:	*Cyprinus carpio*
Common name:	Common carp
Length:	Mean body total length 4.9 ± 0.4 cm (mean ± SD)
Body weight:	Mean body wet weight 1.8 ± 0.5 g (mean ± SD)
Source:	[redacted]
Acclimation period:	48 h (fish were observed for 14 days prior to testing)
Feed:	Commercial trout food ([redacted]) during acclimation period
Starvation:	Animals were starved 48 h before and during the study
Biomass loading:	0.68 g fish/ L test medium

B. Study design and methods

1. In life dates: June 20 to October 27, 2011

2. Experimental treatments:

Based on a range finder test (non-GLP), the definitive test concentration was set at 100 mg test item/L (limit test). The test media with the different test concentrations were prepared by weighing an adequate amount of the test item and dissolving it in a part of the test water, before pouring it back into the test vessels.

The aquaria used were made of glass (h × w × d = 38 x 32 x 36 cm). The test volumes amounted to 40 L. For every test concentration two aquaria were used and were labeled with a study number, a number and the nominal concentration of the test item. The aquaria were placed in a temperature controlled room.

Immediately prior to the test, water samples were taken from the center of the aquaria for analytical determination of the active ingredient concentration. At the start of the test, thirty fish were randomly introduced.

Thirty fish (fifteen fish per test vessel I and II) were exposed in a limit test for 96 h under static test conditions to a nominal concentration of 100 mg a.i./L against a water control (control I and II with 15 fish each) and a solvent control with further 30 fish (separate study).

3. Observation and measurements:

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

Within the study the pH-value, the oxygen saturation level and the temperature were measured with commercial measurement devices, daily.

Recoveries of BYI 02960 were measured in all test levels on day 0, day 2 and day 4 of the exposure period to confirm nominal concentrations.

RESULTS AND DISCUSSION

A. Environmental Parameters

Reconstituted water was used during the acclimation period and for the test. It was prepared by adding salt stock solutions to demineralized water.

Photoperiod:	16 h light : 8 h darkness
Test water:	
Hardness:	40 – 60 mg $CaCO_3$/L
Dissolved oxygen (DO):	> 60% oxygen saturation
pH:	> 6.0 and < 8.0
Water temperature:	Range of 20°C - 24°C
Conductivity:	< 0.2 mS/cm

B. Analytical Findings

Dissolved oxygen concentrations ranged from 81% to 112% oxygen saturation, the pH values ranged from 6.8 to 7.4 and the water temperature ranged from 21.1°C to 24.0°C in all aquaria over the whole testing period.

The analytical determination of BYI 02960 (in water by HPLC – MS / MS) revealed mean recovery values of 99% to 100% of nominal over the whole testing period of 96 hours at the limit test concentration of 100 mg a.i./L. Therefore all results are given as nominal values.

C. Biological Findings

There were neither any sub-lethal effects nor any mortality in the control group.

Cumulative mortality was observed as follows [with a total number of 30 (15 I + 15 II)]:

Table 10.2.2.1- 2: Cumulative Mortality and Behavioral Observations (Total number of fish tested at each concentration: 30 (15 I + 15 II)

Exposure time	4 h		24 h		48 h		72 h		**96 h**	
test item (a.i) [mg / L]	no. of dead	% dead	no. of dead	% dead	no. of dead	% dead	no. of dead	% dead	**no. of dead**	% dead
Control I	0	0	0	0	0	0	0	0	**0**	**0**
Control II	0	0	0	0	0	0	0	0	**0**	**0**
585 (100) I	0	0	0	0	0	0	0	0	**0**	**0**
585 (100) II	0	0	0	0	0	0	0	0	**0**	**0**

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The highest concentration which did not result in any mortality within the exposure period (NOLEC) was 585 (100) mg test item (a.i.)/L. There were no sub-lethal effects noted in the treatment groups, as such the no-observed-effect-concentration (NOEC) was at or greater than 585 (100) mg test item (a.i.)/L.

D. Validity Criteria

Test conditions met all validity criteria, given by the mentioned guidelines. There was less than 5% mortality within the 48-hour settling-in period and ≤ 10% mortality in the controls. Dissolved oxygen saturation was greater or equal to 60% throughout the test and pH variation was ≤ 1.0 units.

E. Biological Endpoints Derived

The acute toxicity of BYI 2960 SL 200 to common carp is summarized as followed:

Test item:	BYI 02960 SL 200 G
Test object:	Common carp (*Cyprinus carpio*)
Exposure:	96h, static, limit test
LC_{50} 96 h:	> 585 (100) mg test item (a.i.) / L
NOEC: highest concentration without toxic effects	≥ 585 (100) mg test item (a.i.) / L
NOLEC: highest concentration causing no mortality	≥ 585 (100) mg test item (a.i.) / L

CONCLUSION

The LC_{50} (96h) of BYI 02960 SL 200 G to Common carp *(Cyprinus carpio)* in a static 96-hour-test was determined to be > 585 (100) mg test item (a.i.)/L. The NOLEC and the NOEC was at or greater than 585 (100) mg test item (a.i.)/L, respectively.

IIIA1 10.2.2.2 Acute toxicity (24 & 48 h) for Daphnia preferably Daphnia magna

Report:	**KIIIA1 10.2.2.2/01; [redacted] T. (2010)**
Title:	Acute toxicity of BYI 02960 SL 200 G to the waterflea *Daphnia magna* in a static laboratory test system
Report No:	EBRVP097
Document No:	M-393738-01-2
Guidelines:	**EPA-FIFRA § 72-2 (1982);** **OPPTS 850.1010 (Public Draft, 1996), modified;** **EEC Directive 92/69/EEC, part C.2 (1992);** **OECD Guideline No. 202 (2004);** **JMAFF, 12 Nousan No. 8147 (2000)**
Deviations:	**None**
GLP	**Yes (certified laboratory)**

Executive Summary

The study was performed to detect possible effects of BYI 02960 SL 200 G (Sample description: TOX 08907-00 (Batch ID: 2010-00106; Material No.: 79718845; Specification No.: 102000021884-01)) on mobility of *Daphnia magna* caused by 48 hours of exposure in a static laboratory test system, expressed as EC_{50} for immobilisation.

Daphnia magna (1st instars < 24 h old, 6 x 5 animals per concentration) were exposed in a static test system for 48 hours to five (geometrically spaced) nominal concentrations of 0, 62.5, 125, 250, 500 and 1000 mg formulation/L, respectively, without feeding. In addition, an untreated dilution water control was tested.

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The content of BYI 02960 in exposure media was measured for verification of the test item concentrations.

No immobilities or other effects on behaviour occurred in the untreated control group within 48 hours of exposure. Based on nominal concentrations of BYI 02960 SL 200 G, EC_{50} values for immobilisation of 1434 and 684 mg formulation/L after 24 and 48 hours of static exposure, were assessed respectively.

MATERIAL AND METHODS

A. Materials

1. Test material:

Test item:	BYI 02960 SL 200 G
Specification number:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Batch No.:	2010-001067
Material number:	79718845
Sample description:	TOX 08907-00
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.1% w/w, 201.0 g/L according to certificate of analysis
Density:	1.175 g/mL at 20 °C
Stability of test compound:	Expiry date: 14.06.2012, when stored at 25 ± 5°C in original container in the dark (also acceptable from +2 to +30°C)

2. Vehicle and/or positive control:

Solvent control:	Water control (negative control)
Positive control:	Toxic reference tested in a separate non-GLP study ($K_2Cr_2O_7$); Result: 24 hour EC_{50} = 0.73 mg/L

3. Test organisms:

Species:	*Daphnia magna*
Common name:	Waterflea
Age at test start:	First instars, < 24 hours old
Source:	Bayer Laboratory stock breeding
Feed:	Living cells of the green alga *Desmodesmus subspicatus*, 3 times per week
Starvation:	Animals were not fed during exposure

B. Study design and methods

1. In life dates: June 7 to 22, 2010

2. Experimental treatments:

Neonates of the waterflea Daphnia magna were exposed for 48 hours to BYI 02960 SL 200 G in aqueous solution without adding any solvents or dispersants.

Any surface in contact with the test solution was made of glass or other chemically inert material.

Exposure occurred in 100 mL glass beakers (DIN 12332), each filled with 50 mL of the test solution, corresponding to a fluid level of approximately 3 cm height.

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Six vessels (replicates), each provided with five daphnids (equivalent to 10 mL test solution per daphnid), were utilised per treatment group and control (corresponding to 30 animals per study group). The beakers were covered with transparent glass plates and placed in a climate controlled environment (isolated chamber) between 18 and 22 °C (maximum allowed deviation ± 1 °C within 48 hours). They were illuminated by "cool white" fluorescent bulbs in a 16:8 hours light-dark cycle, at a light intensity of max. 1200 lux.

The water fleas were not fed and the test solutions were not artificially aerated during exposure.

The study covered five geometrically spaced nominal concentrations (62.5, 125, 250, 500 and 1000 mg form./L = spacing factor 2.0), supplemented by an untreated dilution water (blank) control.

Preparation of test solutions occurred immediately before the start of exposure. Appropriate amounts of the test substance were admixed directly to the test water (Elendt M7) to establish the nominal test concentrations.

The test item showed no remarkable appearance after homogeneous distribution in the test media (clear uncoloured fluid).

3. Observation and measurements:

After 24 and 48 hours, behaviour of the water fleas was visually evaluated by counting mobile daphnids, defined as animals with swimming movements (slight movements of antennae were not interpreted as swimming movement) within approximately 15 seconds after gentle agitation of the test vessel. Additionally all visible features of the test item in water as well as possible signs on sublethal affected daphnids had to be recorded.

Prior to test initiation, conductivity, total hardness, pH and alkalinity of the dilution media (Elendt M7) were determined. Additionally, the dissolved oxygen and pH values were measured in the freshly prepared test solutions of each treatment level and control and in media from the pooled replicates at test termination (day 2).

Light intensity was measured at start of the study as „diffuse light", immediately above the water surface of the test vessels.

Environmental air temperature was continuously recorded during the test by a computer controlled measurement system. Additionally, temperature of the test media was measured inside one vessel of the untreated control and of the highest test concentration at start and end of exposure.

For analytical verification of the nominal exposure concentrations, the content of the active substance BYI 02960 in the exposure solutions was determined. For this purpose, water-samples from start and end of exposure were retained for analysis.

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RESULTS AND DISCUSSION

A. Environmental Parameters

Photoperiod:	16 h light : 8 h darkness
Test water:	
Hardness:	249 mg $CaCO_3$/L
Dissolved oxygen (DO):	8.7 mg/L (~ 100%)
pH:	7.9
Water temperature:	20.6°C – 21.2°C
Conductivity:	582 µS/cm

B. Analytical Findings

Dissolved oxygen concentrations ranged from 100.7% oxygen saturation at test start to 100.4% at the end of the test, the pH values ranged from 7.5 to 7.8 and the water temperature ranged from 20.6°C to 21.2°C in all test vessels over the whole testing period.

The analytical determination of BYI 02960 revealed mean recovery values of 103% and 104% of nominal for freshly prepared and aged solutions (after 48 hours), respectively. Therefore all results are given as nominal values.

C. Biological Findings

No immobilities or other effects on behaviour occurred in untreated control within 48 hours of exposure.

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Table 10.2.2.2- 1: Daphnid toxicity after 48 hours of exposure

nominal test concentration (mg form./L)	replicate No.	immobilised daphnids		mobile daphnids		
				total	affected	
		n / repl.	sum (%)	n / repl.	n / repl.	(type ID)
control (A)	1	0	0.0	5	0	
	2	0		5	0	
	3	0		5	0	
	4	0		5	0	
	5	0		5	0	
	6	0		5	0	
62.5 (A)	1	0	0.0	5	0	
	2	0		5	0	
	3	0		5	0	
	4	0		5	0	
	5	0		5	0	
	6	0		5	0	
125 (A)	1	0	6.7	5	0	
	2	1		4	0	
	3	0		5	0	
	4	0		5	0	
	5	1		4	0	
	6	0		5	0	
250 (A)	1	1	30.0	4	0	
	2	1		4	0	
	3	1		4	0	
	4	1		4	0	
	5	2		3	2	(1)+(6)
	6	3		2	0	
500 (A)	1	0	33.3	5	5	(1)+(6)
	2	2		3	3	(1)+(6)
	3	1		4	3	(1)+(6)
	4	3		2	2	(1)+(6)
	5	3		2	1	(1)+(6)
	6	1		4	3	(1)+(6)
1000 (A)	1	3	63.3	2	2	(1)+(6)
	2	4		1	0	
	3	3		2	2	(1)+(6)
	4	3		2	2	(1)+(6)
	5	4		1	1	(1)+(6)
	6	2		3	3	(1)+(6)

Observed effects definition: (1) Quick, trembling antennae movements. (6) Animals lying on the bottom of the testing vessel. Appearance of the testsolutions: (A) no remarkable observations, clear media

D. Validity Criteria

Test conditions met all validity criteria, given by the mentioned guidelines. There was less than 10% control mortality.

E. Biological Endpoints Derived

Based on nominal concentrations of BYI 02960 SL200G, the following EC50 values for immobilisation after 24 and 48 hours of static exposure were assessed:

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Statistical results of probit analysis conducted for determination of EC_{50} values:

probit analysis for data obtained after	Slope function (after Litchfield & Wilcoxon) ①)	EC_{50} mg form./L (nominally)	lower 95% cl mg form./L (nominally)	upper 95% cl mg form./L (nominally)
24 hours	5.44	**1434**	548	3751
48 hours	3.66	**684**	464	1010

①) The slope function after Litchfield & Wilcoxon is derived from the slope, b (1.36 [24h.], 1.78 [48h.]) of the linearised probit function and computed as $S = 10^{\frac{1}{b}}$ [ref.];
Small values refer to a steep dose/response relation and large ones to a flat relation.

CONCLUSION

Based on nominal concentrations of BYI 02960 SL 200 G, the following EC_{50} values for immobilisation after 24 and 48 hours of static exposure were assessed:

EC50 (24 h)	**1434** form./L
EC50 (48h)	**684** form./L

IIIA1 10.2.2.3 Effects on algal growth and growth rate

Report:	**KIIIA1 10.2.2.3/01; [redacted] E. (2010)**
Title:	*Pseudokirchneriella subcapitata* growth inhibition test with BYI 02960 SL 200 G
Report No:	EBRVP095
Document No:	M-397244-01-2
Guidelines:	**OECD Guideline 201 (2006)**
Deviations:	**None**
GLP	**Yes (certified laboratory)**

Executive Summary

The aim of the study was to determine the influence of the test item on exponentially growing *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and ECx for growth rate of algal biomass (cells per volume). The surrogate for biomass was cell density (used as response parameter), measured by direct counting of algae cells per volume or indirect by calculation of cell numbers after measurement of optical cell density.

Pseudokirchneriella subcapitata were exposed in a chronic multi-generation test for 3 days under static exposure conditions to nominal concentrations of 49.4, 74.1, 111, 167 and 250 mg BYI 02960 SL 200 G/L (Sample description: TOX 08907-00 (Batch ID: 2010-00106; Material No.: 79718845; Specification No.: 102000021884-01)) , respectively, in comparison to an untreated control.

Test conditions met all validity criteria, given by the mentioned guideline. All results are based on nominal test concentrations of the formulation.

The (0 - 72h)-ErC50 for BYI 02960 SL 200 G is > 250 mg form./L (≡ highest concentration tested) and the (0 - 72h) - NOErC is ≥ 250 mg form./L.

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MATERIAL AND METHODS

A. Materials

1. Test material:

Test item:	BYI 02960 SL 200 G
Specification number:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Batch No.:	2010-001067
Material number:	79718845
Sample description:	TOX 08907-00
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.1% w/w, 201.0 g/L
Density:	1.175 g/mL at 20 °C
Stability of test compound:	Expiry date: 14.06.2012, when stored at 25 ± 5°C in original container in the dark (also acceptable from +2 to +30°C)

2. Vehicle and/or positive control:

Solvent control:	Untreated test water (negative control)
Positive control:	Reference tests with 3,5-dichlorophenol or potassium dichromate are conducted event driven. Tests are documented together with strain protocols

3. Test organisms:

Species:	*Pseudokirchneriella subcapitata*
Common name:	Freshwater microalgae
Type:	Strain SAG 61.81
Source:	[redacted]
Test medium:	Mixture of nutrient medium, inoculated algae cells and test item

B. Study design and methods

1. <u>In life dates:</u> 23 July to 11 November, 2011

2. <u>Experimental treatments:</u>

The range of test concentrations was selected based on a pre-experiment in order to define the NOE$_r$C, LOE$_r$C and E$_r$C$_x$ (to cover preferably the range up to 75% growth rate inhibition).

Pseudokirchneriella subcapitata were exposed in a chronic multi-generation test for 3 days under static exposure conditions to nominal concentrations of 49.4, 74.1, 111, 167 and 250 mg formulation/L, respectively, in comparison to an untreated control. The test item was applied into the test medium (a mixture of nutrient medium and inoculated algae cells) on day 0. Three replicate vessels per test level and 6 replicate vessels per control were used. Each replicate contained 150 mL test medium.

To ensure that the algae used as inoculum were exponentially growing, a pre-culture was prepared 4 days before the start of the test and cultivated under the same conditions as in the main test. In order to reach an initial cell density of 10,000 cells/mL in the test medium at the beginning of the 72 hours exposure period of the main test, an adequate dilution of the pre-culture was done with nutrient medium.

The surrogate for biomass was cell density (used as response parameter), measured by direct counting of algae cells per volume or indirect by calculation of cell numbers after measurement of optical cell density.

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3. Observation and measurements:

Morphological examinations of cells using a microscope were made over the exposure period on each study day. Cell numbers per volume (as a surrogate for biomass per volume) were estimated photometrically.

Because all test vessels were placed under isothermal conditions, the temperature was determined by a continuous measurement in one additional incubated glass vessel filled with the same amount of deionised water as in the test vessels. Temperature data was recorded by a data logger that calculated the mean, min and max temperatures (based on continuously (hourly means) measured values).

The pH was measured at each observation time in all test levels and the control by an electronic pH meter. Quantitative amounts of BYI 02960 were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

RESULTS AND DISCUSSION

A. Environmental Parameters

Photoperiod:	Permanent light
pH:	Range 7.8 - 8.4
Water temperature:	22° ± 2°C
Light intensity:	Mean 8,543 lux

B. Analytical Findings

The pH values ranged from 7.9 to 8.4 in the controls and the incubation temperature ranged from 21.5°C to 22.3°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 8543 lux.

The analytical findings of BYI 02960 in the treatment levels found on day 0 were 101% to 107% of nominal (average 104%). On day 3 analytical findings of 101% to 103% of nominal (average 102%) were found. Given that the toxicity cannot be attributed to any of the a.i. compounds but to the formulation as a whole, all results are based on nominal test concentrations of the formulation.

C. Biological Findings

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

Table 10.2.2.3-1: Algae growth after 72 hours

nominal concentration [mg form./L]	cell number after 72 h (means) per mL	(0-72h)-average specific growth rates [days^{-1}]	inhibition of average specific growth rate [%]
control	703 000	1.418	--
49.4	672 000	1.402	1.1
74.1	670 000	1.402	1.1
111	701 000	1.416	0.1
167	712 000	1.421	-0.3
250	700 000	1.416	0.1

test initiation with 10 000 cells/mL

-% inhibition: increase in growth relative to the control

D. Validity Criteria

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

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- 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 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

The static 72 hour algae growth inhibition test with BYI 02960 SL 200 G on *Pseudokirchneriella subcapitata* provided the following effects:

ErC50 (0-72 h)	> 250 mg form./L
NOErC (0-72 h)	≥ 250 mg form./L

CONCLUSION

In a 72 hour growth test with the formulation BYI 02960 SL 200 G on *Pseudokirchneriella subcapitata*, the following endpoints have been determined:

ErC50 (0-72 h)	> 250 mg form./L
NOErC (0-72 h)	≥ 250 mg form./L

IIIA1 10.2.2.4 Marine or estuarine organisms acute toxicity LC_{50}/EC_{50}

The product BYI 02960 SL 200 is not intended to be used in the marine environment. Exposure of salt water species is therefore negligible and tests for the preparation of marine species are not required. Acute tests on some marine species are available for the active substance from which no indication for a greater sensitivity in comparison to freshwater organisms has been obtained (see also IIIA, 10.2.7 to 10.2.11)

IIIA1 10.2.2.5 Marine sediment invertebrates, acute toxicity LC_{50}/EC_{50}

The product BYI 02960 SL 200G is not intended to be used in the marine environment. Exposure of salt water species to the formulated product is therefore negligible and tests for the preparation of marine species are not required. Studies with the active substance are available for the marine shrimp, *Americamysis bahia*, suggesting lower sensitivity than observed for freshwater invertebrates. The toxicity of the active ingredient BYI 02960 to the sediment dwelling organism *Chironomus riparius* has been examined, both on acute and chronic scale, and a risk assessment is provided showing acceptable risk for the use of BYI 02960 SL 200 on agricultural fields.

IIIA1 10.2.3 Microcosm or mesocosm study

No model ecosystem studies, such as microcosms or mesocosms have been performed with BYI 02960 SL 200. The risk assessment to aquatic organisms has shown that by the use of appropriate risk mitigation measures a safe use of the product can be demonstrated ion the basis of tier 1 studies.

IIIA1 10.2.4 Residue data in fish (long term)

BYI 02960 is an insecticide of high water solubility (3.2 g/L), a low log P_{OW} of 1.2 indicates no relevant tendency for concentration of residues from water into fish. A log P_{OW} > 3 is the recommended trigger for further investigations on residues in fish, therefore, residues in fish are considered to be of no concern and no further studies are deemed necessary.

IIIA1 10.2.5 Chronic fish toxicity data

Formulations will not remain intact for the long-term when they reach surface waters, hence, a chronic exposure to the product will not occur. From the acute toxicity data there was no indication that the formulation results in a change of the toxicity determined with the active substance alone. Hence, further testing with the formulated product was not justifiable.

IIIA1 10.2.5.1 Chronic toxicity (28 day exposure) to juvenile fish

Please refer to point IIIA 10.2.5.

IIIA1 10.2.5.2 Fish early life stage toxicity test

Please refer to point IIIA 10.2.5

IIIA1 10.2.5.3 Fish life cycle test

Please refer to point IIIA 10.2.5

IIIA1 10.2.6 Chronic toxicity to aquatic invertebrates

Formulations will not remain in their composition on long-term when they reach surface waters, hence, a chronic exposure to the product will not occur. From the acute toxicity data on *Daphnia magna* and the chronic toxicity data of the formulation on *Chironomus riparius* there was no indication that the formulation results in a change of the toxicity determined with the active substance alone. Hence, further testing with the formulated product was not justifiable.

IIIA1 10.2.6.1 Chronic toxicity to Daphnia magna (21-day)

Please refer to point IIIA1 10.2.6.

IIIA1 10.2.6.2 Chronic toxicity for a representative species of aquatic insects

Report:	**KIIIA1 10.2.6.2/01; [redacted] G., 2011**
Title:	*Chironomus riparius* 28-day chronic toxicity test with BYI 02960 SL G in a water-sediment system using spiked water
Report No:	EBRVP182
Document No:	M-416145-01-2
Guidelines:	**OECD Guideline No. 219 (2004)**
Deviations:	**None**
GLP:	**Yes (certified laboratory)** **The quality of the sediment is checked at least once a year (non-GLP data) for contaminants (e.g. heavy metals)** **The quality of the deionised water is checked at least twice (non-GLP data) a year for residues and contaminants (e.g. pesticides and heavy metals).**

Executive summary

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The aim of the study was to determine the influence of BYI 02960 SL 200 G (Sample description: TOX 08907-00 (Batch ID: 2010-00106; Material No.: 79718845; Specification No.: 102000021884-01); content of active ingredient: 17.1% w/w) on emergence and development of Chironomus riparius for 28-days in a static water-sediment-system (spiked water exposure), expressed as NOEC, LOEC and ECx for emergence rate and development rate, if possible.

First instar of Chironomus riparius larvae, 4 beakers per test concentration and control with 20 animals each) were exposed in a static test system for 28 days to nominal concentrations in the overlying medium (spiked water application) of 8.77, 17.5, 35.1, 70.2, 140 and 281 µg form./L, respectively (corresponding to 1.50, 3.00, 6.00, 12.0, 24.0 and 48.0 µg a.i./L, respectively) of a water-sediment system. In addition, a negative control was tested.

Recoveries of BYI 02960 were measured in the overlying water and pore water of the sediment at 1 hour, 7 days and 28 days after application, respectively, in one additional test container of each nominal test concentration.

Results are expressed as nominal test concentrations of the formulated product and measured initial concentrations of BYI 02960 in the overlying water.

After 28 days of exposure, a NOEC of 70.2 µg prod./L was determined for the development rate and the emergence rate of male and female midges (pooled sex) of the aquatic insect Chironomus riparius.

The LOEC and NOEC (both for emergence rate and development rate, respectively) expressed for the active ingredient BYI 02960 were 24 µg a.i./L (measured initial) and 12 µg a.i./L (measured initial), respectively.

MATERIAL AND METHODS

A. Materials

1. Test material:

Test item:	BYI 02960 SL 200 G
Specification number:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Batch No.:	2010-001067
Material number:	79718845
Sample description:	TOX 08907-00
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.1% w/w, 201.0 g/L
Density:	1.175 g/mL at 20 °C
Stability of test compound:	Expiry date: 14.06.2012, when stored at 25 ± 5°C in original container in the dark (also acceptable from +2 to +30°C)

2. Vehicle and/or positive control:

Solvent control:	Untreated test water (negative control)
Positive control:	None. However, reference tests are done periodically with 3,5-dichlorophenol to show sensitivity of test organisms

3. Test organisms:

Species:	*Chironomus riparius*
Common name:	Aquatic insect, midge
Age:	First instar (L1) of *Chironomus riparius* larvae
Source:	[redacted]
Feed:	Commercial ornamental fish food extract (Tetra Phyll®) 3 times per week during the study
Culture medium:	M7-medium, based on de-ionised water is used as breeding water
Acclimation:	For acclimation and equilibration the test vessels were prepared 7 days before test start
Test system:	Artificial sediment (75% quartz sand, 4% sphagnum moss peat, 20% kaolinite, 0.1% calcium carbonate and 48% deionized water
Sediment layer:	1.5 cm layer of wet sediment at bottom of test vessel

B. Study design and methods

1. In life dates February 4 to July 18, 2011

2. Experimental treatments

Each treatment level has 4 replicates (each consisting of 20 larvae) for biological evaluations. Additional replicates for all test concentrations and the control were used for chemical analysis of the test item on day 0 and day 7 (with chironomids).

The bottom of the test vessels (0.6 L glass beakers, Ø 9.5 cm) was covered with a 1.5 cm layer of wet, artificial sediment. M7-medium based on de-ionized water was added as test water (height of water column = 6.0 cm).

One day prior to treatment (= day -1), the test organisms (L1-larvae) were transferred in a randomised procedure into the test vessels (collectives of five larvae each). The test item was applied to the water on day 0 (test start).

During the study the larvae were fed at least about three times per week with a commercial ornamental fish food extract.

3. Observation and measurements:

Measurements of the water temperature were done continuously in one negative control vessel and recorded hourly by a data logger. Additionally, the temperature was measured once a week in the overlying water of the additional test vessels of each test concentration incl. control(s).

Dissolved oxygen was measured twice per week in the overlying water of the additional test vessels of each test concentration incl. control(s) and additionally in all test vessels at the end of the test (day 28).

The pH was measured once per week in the overlying water of the additional test vessels of each test concentration incl. control(s) and additionally in all test vessels at the end of the test (day 28).

Recoveries of BYI 02960 were measured in the overlying water and pore water of the sediment at 1 hour, 7 days and 28 days after application in one additional test container of each nominal test concentration of 8.77, 17.5, 35.1, 70.2, 140 and 281 µg form./L and control (corresponding to 1.50, 3.00, 6.00, 12.0, 24.0 and 48.0 µg a.i./L).

The test vessels were observed at least 3 times per week to make 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.

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4. Statistical analysis

ECx values (e.g. x = 15, 50) and confidence intervals after 28 days were calculated by probit (or logit, weibit, etc.) analysis or in case of failure by non parametric-methods from the appropriate parameters (endpoints), using a commercial program.

The NOEC and LOEC determinations from the appropriate parameters (endpoints) were done, using the ANOVA procedure (α = 0.05, one sided) and properly selected multiple t-tests a commercial program. In case of a limit test (comparison of control and one treatment group only) the STUDENT t-test can be used.

Calculations were carried out using Microsoft Excel® spreadsheets. All further statistical evaluations were done using the commercial program ToxRat Professional version 2.10.05 (released 20.02.2010).

RESULTS AND DISCUSSION

A. Environmental Parameters

Photoperiod:	16 h light : 8 h darkness
Light intensity	500 – 1000 lux
Dissolved oxygen (DO):	> 81.9% oxygen saturation (7.4 mg O_2/L)
pH:	8.4 – 8.6
Water temperature:	20.3 – 20.6°C
Aeration:	Aeration during equilibration phase. Aeration stopped for 24 h after insertion of test organisms and re-started just before application of test item

B. Analytical Findings

Analysis of the overlying water at the beginning of the exposure period (one hour after spiking) reflect high recoveries of BYI 02960 with 101% to 112% (mean 109%) of nominal concentrations across all test levels. Results are expressed as nominal test concentrations of the formulated product and measured initial concentrations of BYI 02960 in the overlying water.

After 7 days of exposure, recoveries in the overlying water of the test concentrations from 77.7% to 84.2% (mean 81.3%) were found, and after 28 days 53.0% to 62.1 % (mean 57.6%).

C. Biological Findings

Start of emergence was on day 13 to 14 for the control and test concentrations from 8.77 to 140 µg form./L (corresponding to 1.50 to 24 µg a.i./L). No emergence could be observed for the highest test concentration of 281 µg form./L (corresponding to 48 µg a.i./L).

87.5% of the inserted (n = 80) larvae maturated to adults in the control after 28 days, fulfilling the guideline requirements.

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Table 10.2.6.2: Influence on emergence and development rate after 28 days (based on initial nominal concentrations of the formulation in the overlying water)

Initial nominal test concentrations µg form./L	Test concentrations µg a.i./L	Number of emerged midges (introduced midges)	Emergence of inserted larvae (pooled sex)			Development rate (1 / d)
			total (%)	male (%)	female (%)	pooled sex
Control	-	70 (80)	87.5	42.50	45.00	0.063
8.77	1.50	73 (80)	91.3	48.75	42.50	0.063
17.5	3.00	69 (80)	86.3	46.25	40.00	0.064
35.1	6.00	73 (80)	91.3	51.25	40.00	0.065
70.2	12.0	71 (80)	88.8	38.75	50.00	0.064
140	24.0	28 (80)	35.0*	23.75	11.25	0.051*
281	48.0	0 (80)	0	-	-	-

*statistical significance (α = 0.05)

The Chi^2-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.

For development rate of male and female midges (pooled) statistical significance was evaluated for the highest test concentration with emergence of 140 µg form./L, resulting in an NOEC of 70.2 µg form./L. LOEC and NOEC expressed for the active ingredient BYI 02960, is 24 µg a.i./L (measured initial) and 12 µg a.i./L (measured initial), respectively (both emergence and development rate, respectively).

D. Validity Criteria

Control emergence started at day 13 to 14 and was 87.5% at study end exceeding the validity criteria of 70%. The recorded physico-chemical parameters (pH, temperature and oxygen content) were within the acceptance range for test validity as specified by the test guideline (OECD 219).

E. Biological Endpoints Derived

Results are given as nominal concentrations for the formulated product (BYI 02960 SL 200 G) and the active substance (a.i.) as well as the analytically confirmed initial concentration of BYI 02960 in the overlying water:

Endpoints	BYI 02960 SL 200 G					
	NOEC	LOEC	EC_{10}	EC_{15}	EC_{20}	EC_{50}
emergence rate (pooled sex) (95 % cl)	70.2	140	68.8 (29.8 – 98.5)	77.2 (37.6 – 109)	84.6 (44.8 – 120)	126 (85.4– 200)
development rate (pooled sex) (95 % cl)	70.2	140	130 (n.d.)	136 (n.d.)	141 (n.d.)	161 (n.d.)

Endpoints	BYI 02960 (a.i.)					
	NOEC	LOEC	EC_{10}	EC_{15}	EC_{20}	EC_{50}
emergence rate, (pooled sex) (95 % cl)	12.0	24.0	11.8 (5.10 – 16.8)	13.2 (6.43 – 18.6)	14.5 (7.66 – 20.5)	21.5 (14.6– 34.2)
development rate, (pooled sex) (95 % cl)	12.0	24.0	22.2 (n.d.)	23.3 (n.d.)	24.1 (n.d.)	27.5 (n.d.)

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Abnormal observations throughout the study (e.g. dead larvae or pupae which failed to show full development and to emerge) were observed only at test concentration of 140 µg form./L. On day 20 one dead pupae were found and on day 22 one dead midge not fully emerged was observed.

If dead adult midges were found on the water surface, this could be caused by the small space between the water surface and the coverage of the beakers.

CONCLUSION

In a 28-day chronic toxicity test with BYI 02960 SL 200 G in a water-sediment system using spiked water, a NOEC of 70.2 µg form./L. was determined for emergence and development rate of male and female midges of the aquatic insect *Chironomus riparius*.

The LOEC and NOEC (for both emergence and development rate) expressed for the active ingredient BYI 02960, are 24 µg a.i./L (measured initial) and 12 µg a.i./L (measured initial), respectively.

IIIA1 10.2.6.3 Chronic toxicity for a repres. species of aquatic gastropod molluscs

Please refer to point IIIA1 10.2.6.

IIIA1 10.2.7 Accumulation in aquatic non-target organisms

BYI 02960 is a substance of low bioaccumulation potential due to the high water solubility (3 g/L) and low Log P_{ow} (1.2). It can therefore be concluded that BYI 02960 is rapidly distributed in the water environment and the tendency for accumulation in biota will be low. Further testing of non-target species is therefore not justifiable.

IIIA1 10.3 Effects on terrestrial vertebrates other than birds

Toxicity of the active substance to terrestrial vertebrates other than birds

The summary of the toxicity profile of the active substance BYI 02960 to mammals is provided in Table 10.3- 1. Details of the studies concerned are provided in the Tier II summary document on the active substance Annex II, Section 3, Points 5.1 to 5.6.

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Table 10.3- 1: Toxicity of BYI 02960 to mammals

Test species	Test design	Ecotoxicological endpoint	Reference
Acute risk assessment endpoint:			
Rat	acute oral, toxic class method	$LD_{50, cut-off}$ 1) 2000 mg a.i./kg bw (4/6 dead at 2000, 0/6 at 300)	[redacted] (2009) M-349992-01-1
Rat	acute oral neurotoxicity study	LD_{50} > 800 mg a.i./kg bw (2/24 dead at 800, 0/24 at 200 and below)	[redacted] (2009) M-415408-01-1
Combined LD_{50} calculation (TOXCALC, probit): 1607 mg/kg bw			
Reproductive risk assessment endpoint:			
Rat reproduction	Dietary exposure over 2 generations	$NOAEL_{maternal}$ 7.8 1) (100 ppm) mg a.i./kg bw/d $NOAEL_{repro}$ 39.2 2) (500 ppm) mg a.i./kg bw/d $NOAEL_{offspring}$ 7.8 1) (100 ppm) mg a.i./kg bw/d	[redacted] (2011) M-417665-01-1
Rat developmental toxicity	Gavage over GD 6-20 (gestation days)	$NOAEL_{maternal}$ 15 mg a.i./kg bw/d $NOAEL_{offspring}$ 50 mg a.i./kg bw/d	[redacted] (2010) M-363938-01-1
Rabbit developmental toxicity	Gavage over GD 6-28 (gestation days)	$NOAEL_{maternal}$ 15 mg a.i./kg bw/d $NO(A)EL_{offspring}$ 40 mg a.i./kg bw/d	[redacted] (2012) M-423559-01-1
Reproductive risk assessment endpoint proposal		**Tier 1: 7.8 mg/kg bw/d** **Tier 2 (refined): 39.2 mg/kg bw/d**	

Mean achieved dose of P & F1 females at 100 ppm (Table 5.6-14 of KIIA 5.6.1/02, [redacted], 2011)
Mean achieved dose of P & F1 females at 500 ppm (Table 5.6-14 of KIIA 5.6.1/02, [redacted], 2011)

Tier 1 risk assessment: Based on the data of the relevant acute oral toxicity studies and reproductive toxicity studies presented in the overview table above, the Tier 1 risk assessment is conducted in section 10.3.1.1 (acute risk assessment) with an acute oral LD_{50} = 1607 mg as/kg bw/d and in section 10.3.1.3 (reproductive risk assessment) with the lowest NOAEL = 7.8 mg/kg/bw.

Tier 2 risk assessment: According to the refinement options provided in the EFSA GD (Section 4.4, Module 4: Reproductive risk assessment for mammals, step 9), the Tier 1 endpoint can be refined after re-examination of the reproductive studies for endpoints relevant for reproductive performance. Effects on other endpoints can be disregarded.

In this context the EFSA GD specifies a number of potentially relevant endpoints to be evaluated, including body weight, behavior and reproductive performance of adults, and observations on offspring (number, weight, development).

A corresponding evaluation for BYI 02960 is presented in

Table 10.3- 3, based on the toxicological studies summarized in more detail in section 5 of this dossier.

If such refinement of the Tier 1 endpoint is considered, the EFSA GD recommends that additional mammalian toxicity studies should also be examined in order to check whether they contain lower NOAELs for relevant endpoints.

Approach taken in this evaluation:

In order to ensure a high level of protection, all repeated dose oral toxicity studies conducted with BYI 02960 in rodents (rat, mouse), rabbits or dogs were evaluated for potentially relevant endpoints (Table 10.3- 2 and

Table 10.3- 3).

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This evaluation included also neurotoxicity studies, in order to account for non-lethal behavioral effects that could become relevant under field conditions.

Since the protection goal of the reproductive risk assessment is the sustainability of potentially exposed wild mammal populations, these studies were evaluated with regard to population level relevant endpoints, i.e. survival, growth, development, behavior and reproductive performance. Other endpoints (e.g. organ weights, physiology, blood chemistry etc.) were not included in the evaluation.

Furthermore, neither very high doses (more than 3-fold above the proposed refined endpoint), nor chronic studies of extreme exposure duration (≥ 1 year) were included in

Table 10.3- 3.

Since effects on females are typically more relevant for small mammal populations than effects on males, the results of this evaluation are presented in

Table 10.3- 3 in the order of increasing achieved doses for females over all evaluated studies. In order to facilitate the comparison of effects at the different dose levels of each study, the letter code in the first column indicates the study ID (letters A-N, as presented in the following overview table), with the respective dosing group indicated by numbers 1-3 (low, mid and high dose level).

Table 10.3- 2: Repeated oral dosing toxicity studies evaluated for selection of the Tier 2 wild mammal reproductive risk assessment endpoint in Table 10.3.3

Study ID	Study type	Report reference
A	Rat 28-d gavage	M-283421-02-1, KIIA 5.3.1/01
B	Rat 28-d dietary	M-297120-01-1, KIIA 5.3.1/02
C	RAT 90-d	M-329048-03-1, KIIA 5.3.2/01
D	RAT 90-d NT (neurotoxicity)	M-410022-01-1, KIIA 5.7.4/01
E	RAT 1-gen (one generation reproduction)	M-394208-01-1, KIIA 5.6.1/01
F	RAT 2-gen (two generation reproduction)	M-417665-01-1, KIIA 5.6.1/02
G	RAT DT (developmental toxicity)	M-363938-01-1, KIIA 5.6.10/01
H	RAT DT (suppl.)	M-425810-01-1, KIIA 5.6.10/02
I	RABBIT DT (developmental toxicity)	M-423559-01-1, KIIA 5.6.11/01
K	MOUSE 28-d	M-294820-01-1, KIIA 5.3.1/03
L	MOUSE 90-d	M-328668-02-1, KIIA 5.3.2/02
M	DOG 28-d	M-312461-01-1, KIIA 5.3.1/04
N	DOG 90d	M-369978-01-1, KIIA 5.3.3/01

Results of this evaluation:

Most prominent in the evaluation of all these repeated oral dosing studies with BYI 02960 (

Table 10.3- 3) were observations of moderate body weight effects. Severity of effects was seen to increase with dose and exposure time.

Within reasonable timeframes of environmental exposures (weeks to months**), effects of ecological relevance on wild mammal population did not occur at dose levels of at least 30-50 mg/kg bw/d.**

In contrast, pronounced reproductive effects of clear ecological relevance did occur at dose levels significantly exceeding 100 mg/kg bw/d (e.g., 14.8% litter size reduction and reduced body weight of > 10% at 120-150 mg/kg bw/d (1800 ppm) in the rat 2-generation study), and only after prolonged exposure in a second generation(F1 females or F2 offspring).

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Moderate effects of bodyweight can usually be considered less relevant in wild mammal risk assessments. Moreover, the results of the 90-d rat study with BYI 02960 showed that bodyweight effects approaching 10% (e.g., 8.9% in males and 9.5% in females at 500 ppm) were reversible after cessation of treatment (recovery phase). Potential exposure of wild mammals to BYI 02960 is transient and short-lived (single application, DT_{50} on foliage ≤ 5 days). Therefore an effect magnitude of ≤ 10% on bodyweight can be considered ecologically acceptable. Body weight effects exceeding this magnitude were not observed at dose levels below 140 mg/kg bw/d (P females: 15.9%), even after prolonged exposure.

Reproductive effects like reduction of litter size were only moderately pronounced and were only observed after long-term exposure in F1 parents and F2 litter (note: not observed in the 1-generation rat reproduction study nor in the first generation of the 2-generation study), indicating reduced risk for reproductive effects under environmentally relevant conditions (low application rate, single application, DT_{50} on foliage < 5 days).

Overall, this evaluation suggests that a dose level of up to **39.2 mg as/kg bw/d** (achieved dose of females at 500 ppm in the 2-gen rat reproduction study) can be proposed as relevant and sufficiently conservative risk assessment endpoint for wild mammal populations:

- no ecologically relevant effects in the parental generation or the F1 litter
- only slight reductions of bodyweight (< 10%) in F1 parents and F2 litter
- body weight reduction by BYI 02960 of ca. 10% was reversible after cessation of dosing.

Severe effects only occurred at dose levels which were 3-4 times higher than 39.2 mg/kg bw/d.

Therefore the refined wild mammal reproductive risk assessment endpoint proposed in this dossier is **39.2 mg as/kg bw/d**, the achieved dose of females at 500 ppm in the 2-gen rat reproduction study.

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Table 10.3- 3: Potentially relevant wild mammal reproductive risk assessment effects of BYI 02960 in repeated oral dosing studies

F: females; M: males; ↑: increase (higher than control); ↓: reduction (lower than control);
P: parental generation M+F; F1: offspring produced by P M+F, F2: offspring produced by F1 M+F
#: number; bw: body weight; FC: food consumption
n.e.: not evaluated (specific study type without reproductive endpoints); empty fields: no effect observed

ID	Species & study type	Dose ppm	mg/kg bw/d males	mg/kg bw/d females	Potentially relevant findings in adults	Potentially relevant findings on reproduction and offspring
D1	RAT 90-d NT	100	5.7	6.9		n.e.
C1	RAT 90-d	100	6.0	7.6		n.e.
I1	RABBIT DT			7.5		
F1	RAT 2-gen	100	6.4-6.6	7.0-7.8		
N1	DOG 90d	400	12	12		n.e.
I2	RABBIT DT			15		
G1	RAT DT			15		
E1	RAT 1-gen	200	14.5	15.8-17.5		
M1	DOG 28-d	500	16	18		n.e.
L1	MOUSE 90-d	100	16	19		n.e.
H1	RAT DT (suppl.)			20		n.e.
H2	RAT DT (suppl.)			30		n.e.
B1	Rat 28-d	500	33.6			n.e.
D2	RAT 90-d NT	500	29.4	34.8		n.e.
C2	RAT 90-d	500	30.2	38.3		n.e.
F2	RAT 2-gen	500	32-32.5	**35.5-39.8**	bw ↓ ≤ 7.8% (F1 F: D_{gest} 0)	bw ↓ ≤ 7.4% (F2 pups)
I3	RABBIT DT			40	FC ↓ ≤ 20%, bw ↓ ≤ 1.7 %	
N2	DOG 90d	1200	33	41	bw ↓ ≤ 9% (M), minimal/slight muscle atrophy	n.e.
G2	RAT DT			50	FC ↓ ≤ 8%, bw ↓ ≤ 1.6 %	
K1	MOUSE 28-d	300	50	59		n.e.
E2	RAT 1-gen	700	50.1	48.8-60.9	bw ↓ ≤ 6.7% (F D_{lact} 14)	bw ↓ ≤ 9.9 % (F1 pups)
A1	RAT 28d		75	75		n.e.
L2	MOUSE 90-d	500	81	96		n.e.
K2	MOUSE 28-d	600	98	122		n.e.
G3	RAT DT			150	FC ↓ ≤ 27%, bw ↓ ≤ 4.2%	bw ↓ 2-3%, indications of slightly delayed fetal development
F3	Rat 2-gen	1800	117.4-122.1	140.2-151.4	bw ↓ ≤ 9.6% (M), ≤ 15.9% (F)	litter size ↓ 14.8% (F2) bw ↓ ≤ 12.5% (F1 & F2 pubs) # estrous cycles ↓ (F1 F)
C3	RAT 90-d	2500	156	168	bw ↓ ≤ 8.9% (M), 9.5% (F), reversible in recovery phase	n.e.
D3	RAT 90-d NT	2500	143	173	FC ↓ ≤ 29% (F, w1); bw ↓ ≤ 10% (M), ≤ 9% (F);	n.e.
E3	RAT 1-gen	2000	147.5	164.4-182.3	bw ↓ F (≤ 12% D_{lact} 13)	bw ↓ ≤ 9.9% (F1 pups)

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Toxicity of metabolites

The toxicological properties of two metabolites of BYI 02960 were investigated in a series of studies and evaluated in more detail in section KIIA 5.8 of this submission. Toxicological studies performed on two other metabolites which are also common metabolites to other agrochemicals are also reported in this section. Only a brief summary of the results of these studies is presented in this section.

Overall, the results indicate that none of the metabolites is more toxic to mammals than the parent, so that the risk assessment for wild mammals can be based on the toxicity and exposure ratios calculated for BYI 02960 a.i.

The metabolite difluoroacetic acid (DFA) is a major soil, water and plant metabolite and is found in the rat ADME study at an amount of around 6%. It is considered a non-relevant metabolite in terms of the EU Guidance document Sanco/221/2000-rev.10 (25 February, 2003). This metabolite was devoid of genotoxic potential; the acute oral LD50 was between 300 and 2000 mg/kg. When DFA was administered via the diet to Wistar rats for at least 14 days at concentrations of 500, 2000 and 8000 ppm (equating approximately to 48, 187 and 745 mg/kg body weight/day, respectively in males and 51, 201 and 800 mg/kg body weight/day, respectively in females), slight body weight decrease was observed in both sexes at 8000 ppm. In a 90-day rat study, DFA was administered in the diet administration to Wistar rats at concentrations of 200, 1000 and 6000 ppm (equating approximately to 12.7, 66.2, 380 mg/kg body weight/day, respectively in males and 15.6, 78.7, 472 mg/kg body weight/day, respectively in females). Mean body weight and food consumption were reduced at 6000 and 1000 ppm, respectively in both sexes but not at 200 ppm. When the NOAEL of 200 ppm DFA is expressed in BYI 02960 equivalents, it equates to 38 and 47 mg/kg/day in males and females, respectively. Therefore, DFA was not more toxic than BYI 02960 after subchronic administration to the rat.

BYI 02960-difluoroethyl-amino-furanone (DFEAF) is a minor plant metabolite. The acute oral LD50 in rats was higher than 2000 mg/kg, indicating that DFEAF is not more toxic than BYI 02960.

BYI 02960-CHMP ((6-chloro-3-pyridyl)methanol) is a plant metabolite. The acute oral rat LD50 was 1842 mg/kg in males and 1483 mg/kg in females. In a 90-day rat study, BYI 02960-CHMP was administered in the diet to Sprague Dawley rats (10/sex/group) at concentrations of 160, 800, 4000 and 20 000 ppm, (corresponding to 9.9, 48.9, 250.1 and 1246.6 mg/kg/day, respectively for males and 11.1, 55.9, 275.9 and 1173.7 mg/kg/day, respectively for females). Mean body weights and mean food consumption were decreased at 20000 ppm in both sexes. Based on eosinophilic intranuclear inclusions in the proximal tubular epithelium of kidneys at 20000 ppm and 4000 ppm, the no observed effects level (NOEL) was 800 ppm (48.9 mg/kg/day) in males, and 4000 ppm (275.9 mg/kg/day) in females, respectively. When the NOEL is expressed in BYI 02960 equivalents, it equates to 97.8 and 551.8 mg/kg/day, respectively. Therefore, BYI 02960-CHMP was less toxic than BYI 02960 after subchronic administration to the rat.

6-CNA (6-chloronicotinic acid) is a major soil and plant metabolite. An acute oral rat toxicity study and an Ames test were performed on this metabolite for the registration of acetamiprid. 6-CNA was not genotoxic and not acutely toxic.

Toxicity of the formulated product

The acute oral toxicity of the formulated product BYI 02960 SL 200 was determined in a study on rats.

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Table 10.3- 4: Mammalian toxicity data of the formulated product BYI 02960 SL 200

Test species	Test design	Ecotoxicological endpoint	Reference
Rat	acute, oral	LD_{50} >2000 mg/kg bw [1)]	[redacted] (2010) M-385422-01-1, KIIIA1 7.1.1/01

[1)] According to OECD Guideline 423, the LD_{50} cut-off of BYI 02960 SL 200 is >5000 mg/kg body weight (equivalent to Category 5 (unclassified) of the GHS)

Selection of endpoints for risk assessment

The selection of mammalian endpoints for risk assessment follows the same principles as described in detail under point 10.1 for birds, i.e. EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009).

Risk Assessment for mammals

The risk assessment procedure for wild mammals follows the same principles as described in detail under point 10.1 for birds, i.e. EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009).

Mammalian generic focal species for Tier 1 risk assessment

The product is intended to be used in hop at an application rate of 1 x 0.150 kg a.i./ha, BBCH 31-75, and in lettuce at an application rate of 1 x 0.125 kg a.i./ha, BBCH 12-49. According to the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009) the following generic focal species have to be addressed in the risk assessment.

Table 10.3- 5: Relevant generic mammalian focal species for Tier 1 risk assessment

Crop	Growth stage (BBCH)	Generic focal species	Representative species	Shortcut value	
				For long-term RA based on RUD mean	For acute RA based on RUD 90th perc.
Hops	≥ 20	Small insectivorous mammal "shrew"	Common shrew	**1.9**	**5.4**
	≥ 40	Small herbivorous mammal "vole"	Common vole	**21.7**	**40.9**
	20 - 39	Small omnivorous mammal "mouse"	Wood mouse	**3.9**	**8.6**
	≥ 40	Small omnivorous mammal "mouse"	Wood mouse	2.3	5.2
Leafy vegetables	≥ 20	Small insectivorous mammal "shrew"	Common shrew	**1.9**	**5.4**
	40-49	Small herbivorous mammal "vole"	Common vole	**72.3**	**136.4**
	≥ 50	Small herbivorous mammal "vole"	Common vole	21.7	40.9
	All season	Large herbivorous mammal "lagomorph"	Rabbit	**14.3**	**35.1**
	10-49	Small omnivorous mammal "mouse"	Wood mouse	**7.8**	**17.2**
	≥ 50	Small omnivorous mammal "mouse"	Wood mouse	2.3	5.2

Bold values were used for Tier 1 risk assessment. Where the same focal species is representative for different BBCH stages, only the worst-case SV values were chosen for risk assessment.

IIIA1 10.3.1 Toxicity exposure ratios for terrestrial vertebrates other than birds

Summary of calculated TER values for mammals

Table 10.3.1-1: Summary of TER_{AC} values

Crop (BBCH)	Generic focal species	Active substance	SV_{90}	TER_A	Assessment step
Hops (≥20)	Small insectivorous mammal "shrew" Common shrew	BYI 02960	5.4	1984	Tier 1
Hops (≥ 40)	Small herbivorous mammal "vole" Common vole		40.9	262	Tier 1
Hops (20 – 39)	Small omnivorous mammal "mouse" Wood mouse		8.6	1246	Tier 1
Lettuce (≥20)	Small insectivorous mammal "shrew" Common shrew	BYI 02960	5.4	2381	Tier 1
Lettuce (40-49)	Small herbivorous mammal "vole" Common vole		136.4	94	Tier 1
Lettuce (All season)	Large herbivorous mammal "lagomorph" Rabbit		35.1	366	Tier 1
Lettuce (10-49)	Small omnivorous mammal "mouse" Wood mouse		17.2	747	Tier 1

Table 10.3.1-2: Summary of TER_{LT} values (reproductive risk assessment)

Crop (BBCH)	Generic focal species	Active substance	SV_m	TER_{LT}	Assessment step
Hops (≥20)	Small insectivorous mammal "shrew" Common shrew	BYI 02960	1.9	52	Tier 1
Hops (≥ 40)	Small herbivorous mammal "vole" Common vole		21.7	4.5	Tier 1
				12.6-63.4	refined
Hops (20 – 39)	Small omnivorous mammal "mouse" Wood mouse		3.9	25	Tier 1
Lettuce (≥20)	Small insectivorous mammal "shrew" Common shrew	BYI 02960	1.9	62	Tier 1
Lettuce (40-49)	Small herbivorous mammal "vole" Common vole		72.3	1.6	Tier 1
				4.5-22.8	refined
Lettuce (All season)	Large herbivorous mammal "lagomorph" Rabbit		14.3	8	Tier 1
Lettuce (10-49)	Small omnivorous mammal "mouse" Wood mouse		7.8	15	Tier 1

Conclusion: According to the presented risk assessment, the risk to mammals from the use of the product in hops and lettuce is acceptable.

IIIA1 10.3.1.1 Acute toxicity exposure ratio (TER_A)

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Tier 1 acute toxicity exposure ratio for mammals

Table 10.3.1.1- 1: Tier 1 acute DDD and TER calculation for mammals

Crop	Generic focal species	LD_{50} [mg/kg bw]	DDD			DDD	TER_A	Trigger
			Appl. rate [kg/ha]	SV_{90}	MAF_{90}			
BYI 02960								
Hops (≥20)	Small insectivorous mammal “shrew”	1607	0.150	5.4	1	0.81	1984	10
Hops (≥ 40)	Small herbivorous mammal "vole"			40.9		6.14	262	
Hops (20 – 39)	Small omnivorous mammal “mouse”			8.6		1.29	1246	
Lettuce (≥20)	Small insectivorous mammal “shrew”	1607	0.125	5.4	1	0.68	2381	10
Lettuce (40-49)	Small herbivorous mammal "vole"			136.4		17.05	94	
Lettuce (All season)	Large herbivorous mammal “lagomorph”			35.1		4.39	366	
Lettuce (10-49)	Small omnivorous mammal “mouse”			17.2		2.15	747	

All TER values for both crops are well above the trigger of 10 for acute exposure. Hence, no unacceptable risk is to be expected from the use of the product according to the intended use pattern.

Acute risk assessment for mammals drinking contaminated water

For further details, reference is made to Point 10.1.1 of this dossier. However, according to EFSA Guidance Document for Birds and Mammals (2009), unlike for birds the scenario of pools formed in leaf axils is not relevant for mammals. Therefore the risk assessment for mammals is limited to the scenario of puddles formed on the ground after application.

The acute risk from water in puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil is covered by the long-term risk assessment under Point 10.3.1.3 of this dossier.

IIIA1 10.3.1.2 Short-term toxicity exposure ratio (TER_{ST})

A short term risk assessment is not required under European requiremtns.

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IIIA1 10.3.1.3 Long-term toxicity exposure ratio (TER_{LT})

Tier 1 reproductive/long-term toxicity exposure ratio for mammals

Table 10.3.1.3- 1: Tier 1 long-term DDD and TER_{LT} calculation for mammals

Crop	Generic focal species	NOAEL [mg/kg bw/d]	DDD				DDD	TER_{LT}	Trigger
			Appl. rate [kg/ha]	SV_m	MAF_m	f_{TWA}			
BYI 02960									
Hops (≥20)	Small insectivorous mammal "shrew"			1.9			0.15	52	
Hops (≥ 40)	Small herbivorous mammal "vole"	7.8	0.150	21.7	1	0.53	1.73	**4.5**	5
Hops (20 – 39)	Small omnivorous mammal "mouse"			3.9			0.31	25	
Lettuce (≥20)	Small insectivorous mammal "shrew"			1.9			0.13	62	
Lettuce (40-49)	Small herbivorous mammal "vole"			72.3			4.79	**1.6**	
Lettuce (All season)	Large herbivorous mammal "lagomorph"	7.8	0.125	14.3	1	0.53	0.95	8	5
Lettuce (10-49)	Small omnivorous mammal "mouse"			7.8			0.52	15	

Bold values do not meet the trigger

The TER_{LT} values for small herbivorous mammals are below the trigger of 5 in the reproductive/long-term risk assessment for both uses, indicating a need for refinement.

Refined risk assessment

The Tier 1 risk assessment resulted in TER_{LT} < 5 values for small herbivorous mammals for both the uses in hops and on lettuce. Therefore a refined risk assessment is developed in this section, based on

I. a new proposal for a more relevant endpoint to be used in the reproductive risk assessment for small herbivorous mammals,

II. measured residue data that allow refinement of the time-weighted average residue concentrations (21-d fTWA) of BYI 02960 on foliage as potential diet of small herbivorous mammals

III. a more realistic evaluation of the focal species exposure scenario (relevance of hop yards and lettuce fields for voles), based on general knowledge from literature and field study results.

IV. Overall evaluation and conclusions from the refined risk assessment

Re-evaluation of the reproductive risk assessment endpoint

As outlined in section 10.3, a targeted evaluation of the repeated dosing toxicity studies in laboratory mammals (Table 10.3-2 and 10.3-3) allows the proposal of a refined reproductive risk assessment for wild mammals at the dose level of 500 ppm in the rat reproduction study, since no effects of ecological relevance were observed up to this dose level. More pronounced - yet still moderate - effects on parameters of clear relevance for wild mammal populations (survival, development, reproductive performance or behaviour) are not observed below dose levels of 120-150 mg/kg bw/d.

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Thus, selection of a refined reproductive risk assessment endpoint at 39.2 mg/kg bw/d still includes a significant margin of safety for wild mammal populations.

Refined DT50 of BYI 02960 on foliage

According to the recommendations of the EFSA GD (2009), the Tier 1 risk assessment has been conducted with a "generic" DT50 of 10 days of BYI 02960 on all food items. However, measured residue decline data (Dossier Section 4, Point 6, KIIIA1 6.3.1/01-04 for lettuce and KIIA1 10.3.3/02 for cereals) are available that allow the calculation of compound-specific DT50 values for BYI 02960.

From the available residue decline data, it is proposed to select substrates and application types that can be considered representative for foliage as food of small herbivorous mammals in lettuce fields (i.e., spray application residue trial results) and in hop yards (spray application residue trials in young cereals as surrogate for wild undergrowth).

With these data, specific DT_{50} values for BYI 02960 were calculated by [redacted] & [redacted] (2012; lettuce: M-428040-01-1, KIIIA 10.3.3/03; cereals: M-428041-01-1, KIIIA 10.3.3/04) according to best practice in environmental modelling. The residue decline data were analysed with the software package KinGUI, which is a standard tool used for kinetic evaluations. In the first place, fits using the single first order (SFO) model were performed. Their quality was assessed visually and also based on statistical significance of the individual parameters. In case of clear biphasic behaviour in the respective residue data, a DFOP fit (double-first order in parallel) was also performed. Also here, visual acceptability and statistical significance were evaluated.

As a result of KinGUI evaluation, 12 trials in lettuce provided SFO – DT50 values and 5 trials provided a DFOP-DT50. In young cereals, 3 trials provided SFO – DT50 values and 1 trial provided a DFOP-DT50.

Half-live (DT50) values from SFO and DFOP cannot be simply mathematically averaged in order to generate an overall DT50 to be used in time-weighted average (TWA) calculations. However results of individual TWA calculations, applying the respective calculation method for either SFO or DFOP DT50 values, can be combined and used to generate an overall TWA value. With this approach the full set of available data can be included in the refined exposure assessment

The TWA values themselves were thus calculated in a second step as follows. First, numerical evaluation of predicted residues in time (with timestep of 0.1 days) was performed, employing the respective kinetic type and the relevant degradation parameter(s) - DT50 value(s) and g-factor (for DFOP). These predicted residues are then used for the 21-d fTWA evaluation per data set and averaged afterwards see Table 10.3.1.3- 2 and Table 10.3.1.3- 3.

Lettuce: overall, 17 residue dissipation trials with BYI 02960 were available for lettuce. In 12 of these trials, the residue dissipation could be considered according to SFO, whilst the remaining 5 trial results needed fitting with DFOP. Since the (single) DT_{50} from SFO fits cannot simply be averaged with the (double) DT_{50} from DFOP fits, calculations of the 21-d f_{TWA} were conducted for each of the trials, and the 17 resulting 21-d f_{TWA} values were combined afterwards. Due to the number of trials in the evaluation it is proposed to use the **geometric mean 21-d f_{TWA} = 0.19** for the refined long-term exposure assessment for small herbivorous mammals in lettuce fields (Table 10.3.1.3- 2).

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Cereals as surrogate for undergrowth in hop yards: overall, 4 residue dissipation trials with BYI 02960 were available for young cereals. In 3 of these trials, the residue dissipation could be considered according to SFO, whilst in the remaining trial results needed fitting with DFOP. Since the (single) DT_{50} from SFO fits cannot simply be averaged with the (double) DT_{50} from DFOP fits, calculations of the 21-d f_{TWA} were conducted for each of the trials, and the 4 resulting 21-d f_{TWA} values were combined afterwards. Due to the number of trials in the evaluation it is proposed to select **the median 21-d f_{TWA} = 0.19** for the refined long-term exposure assessment for small herbivorous mammals feeding on undergrowth in hop yards (Table 10.3.1.3- 3).

Table 10.3.1.3- 2: DT_{50} and 21-d f_{TWA} for residue dissipation of BYI 02960 on lettuce

Trial code	Trial description	SFO DT_{50}	DFOP DT_{50fast}	DFOP DT_{50slow}	21-d f_{TWA}
R01	10-2223-01, N-EU	2.27	-	-	0.16
R02	10-2223-02, N-EU	-	0.06	7.09	0.37
R03	10-2223-03, N-EU	3.34	-	-	0.23
R04	10-2223-04, N-EU	5.45	-	-	0.35
R05	10-2223-05, N-EU	-	0.16	12.33	0.52
R06	11-2082-01, N-EU	3.04	-	-	0.21
R07	11-2082-02, N-EU	0.20	-	-	0.01
R08	11-2082-04, N-EU	-	0.23	3.91	0.24
R09	10-2213-01, S-EU	-	0.06	3.57	0.21
R10	10-2213-02, S-EU	5.25	-	-	0.34
R11	10-2213-03, S-EU	1.61	-	-	0.11
R12	10-2213-04, S-EU	5.15	-	-	0.33
R13	10-2213-01, S-EU	1.77	-	-	0.12
R14	11-2071-01, S-EU	3.91	-	-	0.26
R15	11-2071-02, S-EU	2.56	-	-	0.18
R16	11-2071-03, S-EU	-	0.95	5.99	0.34
R17	11-2071-04, S-EU	1.31	-	-	0.09
Geometric mean 21-d f_{TWA}					**0.19**

Table 10.3.1.3- 3: DT_{50} and 21-d f_{TWA} for residue dissipation of BYI 02960 on young cereals

Trial code	Trial description	SFO DT_{50}	DFOP $DT_{50\ fast}$	DFOP $DT_{50\ slow}$	21-d f_{TWA}
R01	11-2958-01, N-EU	-	0.193	2.961	0.18
R02	10-2958-02, N-EU	1.4	-	-	0.10
R03	10-2958-03, N-EU	4.08	-	-	0.27
R04	10-2958-04, S-EU	2.98	-	-	0.20
Median 21-d f_{TWA}					**0.19**

Both residue data sets result in 21-d f_{TWA} values of 0.19. These 21-d f_{TWA} factors can be used in the refined quantitative exposure and risk assessment for small herbivorous mammals that may be exposed to residues from application of BYI 02960 in hop yards or lettuce fields.

I. Relevance of the "vole" scenario in hops and lettuce fields

The risk to wild mammals from the use of BYI 02960 has been evaluated according to the Tier 1 scenarios provided in the EFSA GD. These scenarios include small insectivorous mammals ("shrew"), small omnivorous mammals ("mouse"), large herbivorous mammals ("lagomorph"), and small herbivorous mammals ("vole").

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Whilst all other scenarios demonstrated adequate margins of safety, even with the worst case settings of a Tier 1 assessment, the scenario "vole" resulted in Tier 1 TER_{LT} values of 4.5 and 1.6 for the uses in hop and lettuce, respectively. These Tier 1 TER_{LT} values for voles exceed a threshold of 1, indicating that some margins of safety exist even under the worst case settings applied at Tier 1. Quantitative refinement elements (refined reproductive risk assessment endpoint 39.23 mg/kg/bw/d; measured residue decline DT_{50} resulting in 21-d f_{TWA} = 0.19) that have been introduced above allow a re-calculation of the Tier 2 TER_{LT} values that include margins of safety clearly in excess of the *a-priori* acceptability trigger of 5 (Table 10.3.1.3- 4).

Thus, a quantitative refinement at the level of individual exposure and risk can be conducted to demonstrate acceptable risk at the level of individual voles.

However, the protection goal in ecotoxicology aims ultimately at protection of populations rather than individuals. Therefore the section below is intended to additionally evaluate the risk to vole populations from exposure to BYI 02960 after application in hop or lettuce fields. This evaluation is based on the well known biology and ecology of Common voles (*Microtus arvalis*) as the representative species "behind" the generic focal species scenario "vole", and complemented with a targeted field study in hop yards.

Exposure of Populations

The optimum or prime habitat of the common vole requires permanent vegetation cover and includes grassland or perennial crops such as alfalfa and clover fields. Common voles also occur in sub-optimal (secondary) habitats such as arable land. However, only prime habitats harbor permanent vole populations and are essential for the survival of common vole populations. Secondary habitats are only transiently populated, with regular population declines up to extinction during agronomic operations (e.g., ploughing or harvest), or over winter.

Wherever the species occurs; the population densities of Common voles vary seasonally, with regular mass occurrences being followed by a population break-down which can even lead to local extinction.

Spring crops like lettuce fields are not populated during winter, would not provide much cover against predation and could thus be colonized only from adjacent prime habitats at times of high population density. Spring sown vegetable fields would therefore not belong to the potential prime habitats of Common voles (e.g. Spitz, 1977[3]).

A field study conducted in hop yards in Germany (KIIIA1 10.3.3/01) also confirms that the treated area does not harbor significant proportions of the local vole populations.

Thus, any effect from exposure of voles in areas treated with BYI 02960 would be of low, if any, importance on the local population level which depends on the voles living outside of the lettuce fields or the hop yards.

Vulnerability of populations:

The failure to demonstrate TER_{LT} > 5 for voles is not a rare event, due to the worst case combination of factors involved in the scenario. Actually, in regard of theoretical exposure of individual animals, the vole scenarios are nearly always the most critical all over the EFSA GD (2009). However, with regard of the vulnerability of vole populations, the vole scenario is probably the least critical.

[3] [redacted] (1977), Le campagnol des champs (*Microtus arvalis* (Pallas)) en Europe. EPPO Bulletin 7:156-175. BCS Edition no. M-228600-01-1

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The Joint Working Group on the Guidance Document on Risk Assessment for Birds & Mammals (SANCO 10997/2009) has already raised the question on the "need for the vole scenario… given the resilience of the vole populations"; i.e. well-known fact that voles are able to recover after large population breakdowns, or despite eradication programs with targeted rodenticide use (for recent evaluations see e.g.. [redacted] 2010 [4], or [redacted] *et al.* 2010 [5]).

This potential for recovery of Common voles is extraordinarily large, due to the ability of Common voles to achieve high population numbers within short periods of time thanks to the reproduction biology of the species (e.g. [redacted] 1979 [6], [redacted] 2003 [7]):

- the potentially highest number of young per litter ranges between 12 and 13. The weight of a litter is about 53% of the females weight
- average number of young per litter in the field is about 7
- gestation period is about 20 days in the field
- successful mating occurs when young females are not yet weaned
- long reproduction period including occasional winter reproduction

The most straightforward application of the conclusion by the Joint Working Group on the Guidance Document on Risk Assessment for Birds & Mammals (SANCO 10997/2009) (questioning the "need for the vole scenario… given the resilience of the vole populations)" would be that the failure to meet the *a-priori* acceptability trigger for long-term exposure of individual voles is not to be considered as problematic as long as the TER trigger for the other wild mammal scenarios reach the respective TER trigger.

This is clearly the case for exposure of wild mammals to BYI 02960.

<u>*Small herbivorous mammals in hops*</u>

A targeted field study has been conducted in hop yards in Germany (KIIIA1 10.3.3/01).

<u>Live trapping</u> revealed that hop yards do not harbor significant proportions of small herbivorous mammals (Common vole, Field vole or Bank vole), as only 0.09% (3 of 3272) of the individuals of the local vole populations was recorded in-field. <u>Radio-tracking</u> confirmed that voles make very little use of hop yards as feeding habitat. Only one of 10 voles (1 of 5 common voles) potentially spent foraging time in a hop yard. This individual was only trapped once, thus considered a disperser rather than part of a local in-field population.

Overall the results of the study do not confirm that the generic focal species scenario of "small herbivorous mammals (vole)" provided in the EFSA GD 2009 would be relevant for hop yards. To the contrary, a very low number of individual voles (< 0.1%) would be exposed on hop yards and the risk on local populations from effects on these individuals would be negligible.

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[4] [redacted] (2010), Rodent outbreaks in Europe: dynamics and damage. In:Singleton et al. (Eds): Rodent outbreaks: Ecology and Impacts, p: 217-233. BCS Edition no: M-427173-01-1

[5] [redacted] (2010), Comparative efficacy of conventional and new rodenticides against Microtus arvalis (Pallas, 1778) in wheat and alfalfa crops. Crop Protection 29: 487-491. BCS Edition no: M-427179-01-1

[6] [redacted] (1979), Zur Problematik der Bekämpfung von Feldmäusen (Microtus arvalis Pall.)) auf Grünland. Zeitschrift für angewandte Zoologie 66: 35-59. BCS Edition no M-419441-01-1

[7] [redacted] (2003), Short-term effects of farming practices on populations of common voles. Agriculture, Ecosystems and Environment 95: 321-325. BCS Edition no. M-415511-01-1

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Small herbivorous mammals in leafy vegetables

The prime habitat of voles is grassland but the species can also be found in cultivated areas. Stable populations can be developed in multi-annual crops, particularly clover/alfalfa fields which perfectly match the species ecological needs. Whilst autumn sown fields might serve as overwintering habitat, spring sown fields would only be colonized later in the season (summer) and only during periods of high population density, then representing rather a "sink" than a "source" habitat for the local populations).

II Summary, overall evaluation and conclusions from the refined risk assessment

Very clearly, neither hop yards nor lettuce fields are prime habitats for local vole populations. Hop yards or lettuce fields might be used merely during migration and dispersion, particularly during periods of high population density in the surrounding prime habitats. The local population does not depend on the reproductive success of individuals that may be exposed to residues from the application of BYI 02960 in hop yards or lettuce fields.

Where individuals of the Common vole exposed to residues from the application of BYI 02960 in hop yards or lettuce fields, the exposure period would be shortlived since residue concentrations were found to decline rapidly from treated lettuce or young cereals (considered a suitable surrogate for grassy undergrowth in hop yards).

Using the measured residue decline to calculate a refined 21-d f_{TWA} = 0.19, the TER_{LT} are above (TER_{LT} = 12.6 in hops) or very close (TER_{LT} = 4.5 in lettuce) even with the most conservative Tier 1 endpoint of 7.8 mg/kg bw/d.

Thus, significant margins of safety can be assigned even to the hypothetical individual Common voles that might be exposed to residues from the application of BYI 02960 in hop yards or lettuce fields. However, the potential for recovery of vole species is very high. In fact, not even targeted rodenticide applications can reduce local populations in suitable habitats for long time. Due to this known resilience of the Common vole populations, the "small herbivorous mammal" scenario can be considered as to require only a lower level of protection than species that are more vulnerable at the population level.

Finally, the limited vulnerability of voles at the population level, and the limited relevance of the reproductive success of hypothetically exposed individuals to the sustainability of the local population, can be considered to further justify application of a refined reproductive risk assessment endpoint.

Evaluation of the repeated dosing oral toxicity profile of BYI 02960 in various species of mammals (rat, mouse, rabbit, dog) reveals that impacts of ecological relevance for the reproductive performance of wild mammals do not occur at least up to the proposed refined reproductive risk assessment endpoint of 39.2 mg/kg bw/d. At this level, only slight (< 10%) effects on body weight of dams and pups were observed, but not before the production of the second generation (i.e., not seen after environmentally relevant exposure durations). Such moderate body weight effects were seen to be reversible after the end of exposure. More severe effects of more clear ecological relevance occur at dose levels of ca. 140-160 mg/kg bw/d, indicating that even the refined reproductive risk assessment endpoint of 39.2 mg/kg bw/d still includes significant margins of safety.

All in all, it can be concluded with sufficient certainty that the risk for small herbivorous mammal populations from the recommended uses of BYI 02960 in hop yards and lettuce fields can be considered as low and acceptable.

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Table 10.3.1.3- 4: Refined reproductive risk assessment for small herbivorous mammals

Crop	Refinement steps	NOAEL [mg/kg bw/d]	DDD Appl. rate [kg/ha]	DDD SV_m	DDD MAF_m	DDD f_{TWA}	DDD	TER_{LT}	Trigger
BYI 02960									
Hops (≥ 40)	Tier 1	7.8	0.150	21.7	1	0.53	1.7	4.5	5
	Refined 21-d f_{TWA}					0.19	0.6	12.6	
	Refined reproductive risk assessment endpoint	39.2				0.53	1.7	22.7	
	Refined 21-d f_{TWA} and refined reproductive risk assessment endpoint					0.19	0.6	63.4	
Lettuce (≥ 20)	Tier 1	7.8	0.125	72.3	1	0.53	4.8	1.6	5
	Refined 21-d fTWA					0.19	1.7	4.5	
	Refined reproductive risk assessment endpoint	39.2				0.53	4.8	8.2	
	Refined 21-d fTWA and refined reproductive risk assessment endpoint					0.19	1.7	22.8	

The refined quantitative risk assessments conducted in Table 10.3.1.3- 4 for the scenario of small herbivorous mammals ("vole") result in TER_{LT} values ranging 12.6 to 63.4 for uses in hop yards and 4.5 to 22.8 for uses in lettuce fields, also confirming that the risk from the recommended uses of BYI 02960 can be considered as low and acceptable.

Long-term risk assessment for mammals drinking contaminated water

For further details, reference is made to Point 10.1.1 of this dossier.

Table 10.3.1.3- 5: Evaluation of potential concern for exposure via drinking water of mammals (escape clause)

Compound	Koc [mL/g]	Application rate * MAF [g a.i./ha]	NO(A)EL [mg a.i./kg bw/d]	Ratio (Application rate * MAF) / NO(A)EL	"Escape clause" No concern if ratio	Conclusion
BYI 02960	98.4 [1)]	150 [2)] x 1	7.8	19.2	≤ 50	No concern

[1)] Arithmetic mean of six K_{OC} values (see Section 5)

[2)] Worst-case application rate for the use in hops

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

IIIA1 10.3.2 Effects on terrestrial vertebrates other than birds

IIIA1 10.3.2.1 Acute oral toxicity of the preparation

The risk assessment (Tier 1 and refined) based on the active substance revealed TER values well above the respective triggers indicating acceptable acute and long-term risks to mammals (see Points 10.3.1.1 and 10.3.1.3 of this dossier).

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Table 10.3.2.1- 1: Toxicological profile of BYI 02960 SL 200 G

Test system	Test species	LD_{50} [mg product/kg bw]	Reference (see IIIA, Point 7)
Acute oral	Rat	> 2000 mg/kg bw [1)]	[redacted] (2010) M-385422-01-1 KIIIA1 7.1.1/01

1) According to OECD Guideline 423, the LD_{50} cut-off of BYI 02960 SL 200 is >5000 mg/kg body weight (equivalent to Category 5 (unclassified) of the GHS)

IIIA1 10.3.2.2 Acceptance of bait, granules or treated seed

Not applicable for spray application.

IIIA1 10.3.2.3 Effects of secondary poisoning

For details regarding log Pow of the active substances and their metabolites refer to IIIA 10.1.9.

The log P_{OW} value for BYI 02960 and its metabolites is below the trigger value indicating a very low risk of secondary poisoning. No risk assessment is necessary.

IIIA1 10.3.3 Supervised cage or field trials or other appropriate studies

Report:	**KIIIA1 10.3.3/01; [redacted] C, (2004)**
Title:	Generic Field Monitoring of Birds and Mammals in Hop-Cultivation in Southern Germany
Report No:	WFC/FS009
Document No:	M-123479-01-1
Guidelines:	**The monitoring was especially designed for the purpose of this study**
Deviations:	**Not applicable**
GLP:	**Yes (certified laboratory)**

Aim of the study

This generic field study was conducted to determine the real focal species for hop yards, and their potential exposure at the population and the individual level. In the study report, investigations on both birds and wild mammals are compiled, but in this dossier the use of the study is limited to the refined risk assessment for small mammal and thus the study summary is accordingly limited to small mammal observations.

The study was conducted in June and July in in the '[redacted]region in Bavaria, Germany. This region is the main area of hop cultivation in Europe. Small mammal observations were made with live trapping and radio-tracking on three hop yards in their surroundings, considered to be typical sites for hop cultivation which provided a high diversity of birds and mammals.

The combination of these complementary methods allows defining the relevance of small mammal species for risk assessments on hop yard applications in two aspects:

1. Relevance of wild mammal species: which species are using hop yards regularly as part of their home range, and by a significant proportion of the local population?

   ➔ relevance at <u>population level</u>

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2. Relevance for individuals: proportion of their potentially foraging time (PT) of individuals within the crop:

   ➔ exposure of individuals

Methods

Live trapping of small mammals with individual marking and subsequent recaptures served to identify those species that used hop yards as part of their natural home range. In each site 45 traps were installed in a U-shape, with two parallel trap lines (one in the hop yard, the other in the surrounding), and a third transect line from the surrounding vegetation into the hop yard.

Each captured animal was individually marked by a Passive Integrated Transponder (PIT). Species, sex, weight, reproductive state, trap location, date and time of trapping were noted.

According to the use of the study results in the refined risk assessment, the trapping results are summarized here with the focus on the proportion of catches that were made in-field.

Radiotracking served to monitor over 24 h the location, habitat, and behaviour of 21 individuals of 5 different species. From the telemetry data the potentially foraging time, the time in habitat, the speed in the different habitats and the home range were calculated.

According to the use of the study results in the refined risk assessment, the trapping results are summarized here with the focus on species identified during the study

Results

Live trapping resulted in 4635 trapping events, with 346 individuals being marked during the study. Five different rodent species (Wood mouse, Yellow necked mouse, Common vole, Field vole and Bank vole) had stable populations at least at one of the study sites. Additionally 4 shrews and one hazel dormouse were trapped.

Only the wood mouse was caught in significant numbers inside the hop yard (37.5% of the total trappings of this species).

Voles were basically trapped only in the surrounding structure. They very clearly preferred the natural habitats instead of the hop yards.

Common voles were numerous and occurred at all 3 sites, but nearly exclusively off-field (only two individual in-field captures out of 1815 trappings). Similarly, Field voles and bank voles occurred in the surroundings of all sites but only one single trapping of these species was inside a hop yard.

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Species	Marked individuals [#]	FO site	FO in-field	% in-field captures [# of total N]
Wood mouse *Apodemus sylvaticus*	74	3/3	3/3	37.5% [121 of 323]
Yellow necked mouse *Apodemus flavicollis*	16	2/3	1/3	0.8% [1 of 134]
Common vole *Microtus arvalis*	142	3/3	1/3	0.1% [2 of 1815]
Field vole *Microtus agrestis*	35	3/3	0/3	0.0% [0 of 466]
Bank vole *Chlethrionomys glareolus*	70	3/3	1/3	0.1% [1 of 991]
Common shrew *Sorex araneus*	4	3/3	0/3	0.0% [0 of 24]
Hazel dormouse *Muscardinus avellanarius*	1	1/3	1/3	66.7% [2 of 3]

FO: frequence of occurrence
FO_{site} # of sites where the species was observed / # all sites
$FO_{in\ field}$: # of sites where the species was observed in the hop yards / # all sites

Radiotracking confirmed that only the Wood mouse regularly uses the hop yards as foraging habitat (mean PT = 0.242, N = 5 individuals who actually used the hop yard). Five common voles were also radio tracked. Of these, only one individual (a fertile male) used the habitat hop yard and spent 100% of the 24 h telemetry in it. However, this individual was only caught once (on the day of radiotracking) in the hop yard and was considered to be on dispersal rather than part of a local population in the hop yard. Apart from this individual common vole who was most of the time (16.32 h) stationary or resting in the hop yard, none of the other common voles (4), field voles (2) or bank voles (3) was using the hop yard during the 24 h of individual radiotracking.

Conclusion

Live trapping revealed that hop yards do not harbor significant proportions of small herbivorous mammals (Common vole, Field vole or Bank vole), as only 0.09% (3 of 3272) of the individuals of the local vole populations was recorded in-field. Radio-tracking confirmed that voles make very little use of hop yards as feeding habitat. Only one of 10 voles (1 of 5 common voles) spent potentially foraging time in a hop yard, and this individual was only trapped once and considered a disperser.

Overall the results of the study do not confirm that the generic focal species scenario of "small herbivorous mammals (vole)" provided in the EFSA GD 2009 would be relevant for hop yards. In contrary, a very low number of individual voles (< 0.1%) would be exposed on hop yards and the risk on local populations from effects on these individuals would be negligible.

Report:	**KIIIA1 10.3.3/02; [redacted] L. (2012)**
Title:	Determination of the residues of BYI 02960 in/on barley and wheat after spray application of BYI 02960 SL 200 in the field in Germany, southern France and Italy
Report No:	11/2958
Document No:	M-427494-01-1
Guidelines:	**EC Guidance working document 7029/VI/95 rev. 5 (1997)**
Deviations:	**None**
GLP:	**Yes (certified laboratory)** **Soil characterization, climatic data & irrigation recording, pesticide history, cultural practices and applications for maintenance were non-GLP records.**

Aim of the study

The purpose of this study was to determine the magnitude of the relevant residues of BYI 02960 in/on barley (sample material green material) and wheat (sample material green material) after one spray application with BYI 02960 SL 200, an SL formulation containing 200 g/L of BYI 02960.

Material and Methods

Test Item Name:	BYI 02960 SL 200 g/L	**Batch No.:**	2010-007173
Formulation Type and Content:	200 SL (SL: soluble concentrate)	**Expiry Date:**	2012-09-30
Analysis Certificate Number:	FAR No.: 01535-00	**Date of Analysis:**	2010-09-30
Active Ingredient (a.i.):	BYI 02960	**Content a.i.: Nominal / Actual**	200 g/L / 198.6 g/L

Experimental Treatments

The application rates of the actives substances were calculated based on the nominal contents. No additional adjuvants, surfactants or mixing partners were used for application.

The relevant residue of BYI 02960 (common name: flupyradifurone) comprises:

- BYI 02960 (parent compound), difluoroacetic acid (DFA) and BYI 02960-difluoroethylaminofuranone (DFEAF), all calculated as BYI 02960. These components are summed up to the total residue of BYI 02960 calc.

The study included four supervised residue trials conducted in Northern Europe (Germany) and Southern Europe (France and Italy) during the 2011 season.

The actual application data are presented in the following table. This data reflects the intended application scheme, or, if minor deviations occurred, these were within the acceptable range:

Table 10.3.3-1: Application summary

				Application					
Trial No. Country	**Formulation**	**Appl. Mode**	**Crop**	**No.**	**Growth Stage (BBCH)**	**Product Rate (L/ha)**	**Water Rate (L/ha)**	**Active Substance**	**Application Rate [kg a.i. /ha]**
11-2958-01 Germany	BYI 02960 SL 200	SPI	barley	1	29	0.625	300	BYI 02960	0.125
11-2958-02 Germany	BYI 02960 SL 200	SPI	wheat	1	25	0.625	300	BYI 02960	0.125
11-2958-03 France	BYI 02960 SL 200	SPI	barley	1	29	0.625	300	BYI 02960	0.125
11-2958-04 Italy	BYI 02960 SL 200	SPI	wheat	1	29	0.625	300	BYI 02960	0.125

a.i.: active ingredient SPI = Spraying

The analyses were conducted according to the following analytical method:

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Table 10.3.3- 2: Summary of analytical method criteria relevant to this study

Active ingredient	Analytes	Method number	Limit of quantitation [mg/kg]	Sample material	Measurement principle
BYI 02960	BYI 02960	01212	0.01*	green material	LC/MS/MS
	difluoroacetic acid	01212	0.02*	green material	LC/MS/MS
	BYI 02960-difluoroethylaminofuranone	01212	0.01*	green material	LC/MS/MS

* Calculated as BYI 02960

Findings

The average recoveries were within the acceptable range of 70 – 110%, except for the LOQ level for difluoroacetic acid at 116%. Nevertheless, the results are considered as valid.

No residues above the LOQ were found in the control samples. Results were not corrected for concurrent recoveries.

The level of residues of BYI 02960 (parent compound), difluoroacetic acid (DFA), BYI 02960-difluoroethylaminofuranone (DFEAF) and total residue of BYI 02960 calc. in the treated samples are summarised in the table hereafter.

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Table 10.3.3- 3: Residue summary in/on barley and wheat (trial 11-2958-01)

Trial No. Country Crop	Sample material	DALT	Residues [mg/kg] BYI 02960	DFA	DFEAF	Total residue of BYI 02960 calc.(1)
11-2958-01 Germany Barley	green material	0	6.9	0.021	0.069	6.9
	green material	1	1.3	0.032	0.093	1.4
	green material	3	0.75	0.036	0.11	0.89
	green material	5	0.57	0.038	0.095	0.71
	green material	7	0.25	0.040	0.068	0.36
	green material	10	0.14	0.034	0.040	0.21
	green material	15	0.042	0.038	0.021	0.10
	green material	21	0.016	0.043	0.010	0.069
11-2958-02 Germany Wheat	green material	0	11	0.021	0.019	11
	green material	1	8.3	0.028	0.044	8.4
	green material	3	1.7	0.071	0.078	1.8
	green material	5	0.66	0.080	0.039	0.78
	green material	7	0.27	0.078	0.017	0.37
	green material	10	0.081	0.060	< 0.01	0.15
	green material	14	0.027	0.056	< 0.01	0.093
	green material	21	0.021	0.072	< 0.01	0.10
11-2958-03 France Barley	green material	0	16	< 0.02	0.024	16
	green material	1	9.8	0.024	0.039	9.8
	green material	3	9.7	0.089	0.10	9.9
	green material	4	8.3	0.096	0.10	8.5
	green material	7	3.8	0.11	0.087	4.0
	green material	10	1.8	0.10	0.042	1.9
	green material	14	1.1	0.11	0.033	1.2
	green material	21	0.25	0.073	< 0.01	0.33
11-2958-04 Italy Wheat	green material	0	6.9	0.034	0.016	6.9
	green material	1	4.7	0.030	0.017	4.7
	green material	3	2.9	0.031	0.055	3.0
	green material	5	2.6	0.050	0.073	2.7
	green material	7	1.5	0.047	0.056	1.6
	green material	10	0.41	0.074	0.033	0.52
	green material	14	0.17	0.065	0.017	0.25
	green material	21	0.071	0.10	< 0.01	0.18

DALT = Days after last treatment

(1) For the calculation of the total residue of BYI 02960 calc. as it appears in the result table above, unrounded values were used. Therefore, minor deviations may occur between the total residue value shown above and when the values given for the individual analytes in the table are summed. In cases in which the residue levels for all analytes are below the respective LOQs in a given sample, the total residue of BYI 02960 calc. is reported as < 0.04 mg/kg (the sum of the individual LOQs). If a residue value is at or above the individual LOQ level for at least one analyte in a sample, all other values below the respective LOQs in that sample are set at the LOQ before the total residue is calculated (0.01 + < 0.02 + < 0.01 = 0.04 mg/kg)

Analyte:	Final determination as:	Residues calculated as:
BYI 02960	BYI 02960	BYI 02960
BYI 02960-difluoroethylaminofuranone	BYI 02960-difluoroethylaminofuranone	BYI 02960
Difluoroacetic acid	Difluoroacetic acid	BYI 02960
Total residue of BYI 02960 calc.		BYI 02960

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Report:	**KIIIA1 10.3.3/03; [redacted]., [redacted] (2012)**
Title:	Statement on residue dissipation of flupyradifurone in treated foliage of lettuce: kinetic evaluation
Report No:	EnSa-12-0154
Document No:	M-428040-01-1
Guidelines:	**None**
Deviations:	**None**
GLP:	**No (calculation)**

Summary

The residue decline data are available from regulatory plant residue studies were kinetically evaluated to determine the half-lives of BYI 02960 on/in green parts of dicotyledonous plants (lettuce) that may represent food items for leaf-eating herbivorous birds or mammals.

The single-first-order (SFO) half-lives for BYI 02906 and for the total residue of flupyradifurone were derived. In accordance with the approach of FOCUS kinetics SFO and biphasic models were tested, for several trials the decline behavior was found to be biphasic and was described by the DFOP model.

Methods

The residue data from regulatory studies on lettuce was analysed kinetically to determine the half-life for use in the ecotoxicological risk assessment for leaf eating birds and mammals. In total for lettuce 17 data sets were available (see Section 4, Point 6, KIIA1 6.3.1/01-04 for lettuce).

SFO models and biphasic models (DFOP) were evaluated to describe the decline kinetics. The best fitting values of the kinetic parameters from either SFO or DFOP were determined by a numerical optimization process. Using non-linear least square fitting algorithms the parameter values leading to the smallest deviations between observed and calculated residues were determined. Apart from the kinetic rates *k* also the initial amount was fitted. Dissipation half-lives (DT_{50}) were calculated from the dissipation rates *k*, as $DT_{50} = \ln(2) / k$. The appropriate model, for each data set, was determined by visual inspection of the fitted data and the chi^2 test as a measure of the errors.

In cases where the original residue data contained the value of <0.01, the following procedure was employed. In the first occurrence the value was replaced by 0.005. The following data should not be used in the fit and the curve should be cut-off after the first occurrence of <0.01 not followed by a positive detect above the limit value.

Results

The results of the fitting procedure for all residue trials with BYI 02960 only and for the total residue of BYI 02960 are summarised in the tables below.

For the trials R02, R05, R08, R09 and R16, the SFO model provided non acceptable visual fits with scaled errors > 20%. The SFO model could therefore not be chosen for derivation of kinetic endpoints for these trials. For these trials the DFOP model provided acceptable visual fits and lower scaled errors.

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Table 10.3.3- 4: DT50 values for BYI 02960 (BYI 02960 only)

Trial code	Trial description	Model selected	DT50 [days]
R01	10-2223-01, N-EU	SFO	2.27
R03	10-2223-03, N-EU	SFO	3.34
R04	10-2223-04, N-EU	SFO	5.45
R06	11-2082-01, N-EU	SFO	3.04
R07	11-2082-02, N-EU	SFO	0.20
R10	10-2213-02, S-EU	SFO	5.25
R11	10-2213-03, S-EU	SFO	1.61
R12	10-2213-04, S-EU	SFO	5.15
R13	10-2213-01, S-EU	SFO	1.77
R14	11-2071-01, S-EU	SFO	3.91
R15	11-2071-02, S-EU	SFO	2.56
R17	11-2071-04, S-EU	SFO	1.31
R02	10-2223-02	DFOP	0.063/ 7.086#
R05	10-2223-05	DFOP	0.159/ 12.323#
R08	11-2082-04	DFOP	0.231/ 3.908#
R09	10-2213-01	DFOP	0.062/ 3.569#
R16	11-2071-03	DFOP	0.949/ 5.989#
#DT_{50} of fast/slow phase of DFOP			

Table 10.3.3- 4: DT50 values for BYI 02960 total residues)

Trial code	Trial description	Model selected	DT50 [days]
R01	10-2223-01, N-EU	SFO	2.08
R03	10-2223-03, N-EU	SFO	3.58
R04	10-2223-04, N-EU	SFO	4.17
R06	11-2082-01, N-EU	SFO	3.25
R07	11-2082-02, N-EU	SFO	0.21
R10	10-2213-02, S-EU	SFO	5.55
R11	10-2213-03, S-EU	SFO	1.68
R12	10-2213-04, S-EU	SFO	5.22
R13	10-2213-01, S-EU	SFO	1.91
R14	11-2071-01, S-EU	SFO	4.40
R15	11-2071-02, S-EU	SFO	2.67
R17	11-2071-04, S-EU	SFO	1.44
R02	10-2223-02	DFOP	0.064 / 7.686#
R05	10-2223-05	DFOP	0.159 / 12.325#
R08	11-2082-04	DFOP	0.264 / 4.514#
R09	10-2213-01	DFOP	0.064 / 3.954#
R16	11-2071-03	DFOP	1.006 / 9.111#
#DT_{50} of fast/slow phase of DFOP			

Report:	**KIIIA1 10.3.3/04; [redacted], [redacted] (2012)**
Title:	Statement on residue dissipation of flupyradifurone in treated foliage of monocotyledonous plants:: kinetic evaluation
Report No:	EnSa-12-0156
Document No:	M-428041-01-1
Guidelines:	**None**
Deviations:	**None**
GLP:	**No (calculation)**

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Summary

The residue decline data are available from regulatory plant residue studies were kinetically evaluated to determine the half-lives of BYI 02960 on/in green parts of monocotyledonous plants (cereals) that may represent food items for leaf-eating herbivorous birds or mammals.

The single-first-order (SFO) half-lives for BYI 02906 and for the total residue of flupyradifurone were derived. In accordance with the approach of FOCUS kinetics SFO and biphasic models were tested, for 3 trials the decline was described by SFO kinetics while in one trial it was best described by the DFOP model.

Methods

The residue data from regulatory studies on cereals was analysed kinetically to determine the half-life for use in the ecotoxicological risk assessment for leaf eating birds and mammals. In total for cereals 4 data sets were available (see KIIIA 6.3.3/02).

SFO models and biphasic models (DFOP) were evaluated to describe the decline kinetics. The best fitting values of the kinetic parameters from either SFO or DFOP were determined by a numerical optimization process. Using non-linear least square fitting algorithms the parameter values leading to the smallest deviations between observed and calculated residues were determined. Apart from the kinetic rates *k* also the initial amount was fitted. Dissipation half-lives (DT_{50}) were calculated from the dissipation rates *k*, as $DT_{50} = \ln(2) / k$. The appropriate model, for each data set, was determined by visual inspection of the fitted data and the chi^2 test as a measure of the errors.

In cases where the original residue data contained the value of <0.01, the following procedure was employed. In the first occurrence the value was replaced by 0.005. The following data should not be used in the fit and the curve should be cut-off after the first occurrence of <0.01 not followed by a positive detect above the limit value.

Results

The results of the fitting procedure for all residue trials with BYI 02960 only and for the total residue of BYI 02960 are summarised in tables below

Table 10.3.3- 4: DT50 values for BYI 02960 (BYI 02960 only)

Trial code	Trial description	Model selected	DT50 [days]
R02	11-2958-02	SFO	1.40
R03	11-2958-03	SFO	4.08
R04	11-2958-04	SFO	2.98
R01	11-2958-01	DFOP	0.153/ 2.961#
#DT_{50} of fast/slow phase of DFOP			

Table 10.3.3- 4: DT50 values for BYI 02960 total residues

Trial code	Trial description	Model selected	DT50 [days]
R02	11-2958-02	SFO	1.44
R03	11-2958-03	SFO	4.22
R04	11-2958-04	SFO	3.15
R01	11-2958-01	DFOP	0.145/ 3.555#
#DT_{50} of fast/slow phase of DFOP			

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IIIA1 10.4 Effects on bees

Acute toxicity of technical BYI 02960 to honey bees

Details of the honey bee testing with the active substance BYI 02960 and relevant metabolites are presented in Annex II, Section 6, Point 8.3.1.

Table 10.4- 1: Acute toxicity of technical BYI 02960 to honey bees in the laboratory

Test substance	Ecotoxicological Endpoint: LD_{50}		Reference
BYI 02960 (tech.)	oral 24 h oral 48 h contact 24 h contact 48 h contact 72 h contact 96 h	1.3 µg a.i./bee **1.2 µg a.i./bee** >200 µg a.i./bee >200 µg a.i./bee 158.4 µg a.i./bee **122.8 µg a.i./bee**	[redacted] & [redacted] (2008) M-308904-02-1 KIIA 8.7.1/01
	Ecotoxicological Endpoint: NOED		
	oral contact	0.68 µg a.i./bee 25 µg a.i./bee	

Bold values: Endpoints considered relevant for risk assessment

Acute toxicity of formulated BYI 02960 to honey bees

The acute oral toxicity of the formulated product BYI 02960 SL 200 G was determined in a study on bees.

Table 10.4- 2: Bee toxicity data of the formulated product BYI 02960 SL 200 G

Test substance	Ecotoxicological Endpoint: LD_{50}		Reference
BYI 02960 SL 200 G	oral 24 h oral 48 h contact 24 h contact 48 h contact 72 h	3.2 µg a.i./bee **3.2 µg a.i./bee** 21.5 µg a.i./bee 17.1 µg a.i./bee **15.7 µg a.i./bee**	[redacted] (2009) M-359920-02-1 KIIIA1 10.4.2.1/01
	Ecotoxicological Endpoint: NOED		
	oral contact	2.6 µg a.i./bee 6.3 µg a.i./bee	

Bold values: Endpoints considered relevant for risk assessment

Acute toxicity of BYI 02960 metabolites to honey bees

In the plant metabolism studies, a range of metabolites have been measured in the reproductive plant organs (flowers). The observed metabolites were tested in acute and chronic toxicity studies for potential effects on honey bees. Table 10.4- 3 provides an overview of the investigated metabolites, including the parent compound.

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Table 10.4- 3: Overview table on names and synonyms of compounds addressed within the bee chapter

Abbreviation / Chemical code	Alternative name
BYI 02960	Flupyradifurone
BYI 02960-DFEAF	BYI 02960-difluoroethyl-amino-furanone
BYI 02960-OH	BYI 02960-hydroxy
DFA	Difluoroacetic acid
6-CNA	6-chloronicotinic acid
BYI 02960 -CHMP	6-chloropicolyl alcohol, 6-CPA

Table 10.4- 4: Acute toxicity of BYI 02960 metabolites to honey bees in the laboratory

Test substance	Ecotoxicological Endpoint		Reference
BYI 02960-DFEAF	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>81.5 µg a.i./bee** **>100 µg a.i./bee** **≥81.5 µg a.i./bee** **≥100 µg a.i./bee**	[redacted], 2010 M-398557-01-2 KIIA 8.7.1/02
BYI 02960-OH	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>105.3 µg a.i./bee** **>100 µg a.i./bee** **≥105.3 µg a.i./bee** **≥100 µg a.i./bee**	[redacted], 2011 M-409606-01-2 KIIA 8.7.1/03
DFA	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>107.9 µg a.i./bee** **>100 µg a.i./bee** **≥107.9 µg a.i./bee** **≥100 µg a.i./bee**	[redacted], 2010 M-367915-01-2 KIIA 8.7.1/04
6-CNA	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>107.1 µg a.i./bee** **>100 µg a.i./bee** **≥107.1 µg a.i./bee** **≥100 µg a.i./bee**	[redacted], 2010 M-395279-01-2 KIIA 8.7.1/05
BYI 02960 -CHMP	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>106.7 µg a.i./bee** **>100 µg a.i./bee** **≥106.7 µg a.i./bee** **≥100 µg a.i./bee**	[redacted], 2010 M-361234-01-2 KIIA 8.7.1/06

Bold values: Endpoints considered relevant for risk assessment

Chronic toxicity of BYI 02960 and its metabolites to adult honey bees

There are currently no harmonised and ring tested test guidelines available to assess the chronic risk to adult honey bees. Nonetheless, there is some experience within the European honey bee testing community on conducting chronic studies in adult honey bees, by exposing honey bees orally to a treated 50% (w/v) sucrose solution as an exclusive food source for a period of 10 consecutive days by continuous and *ad libitum* feeding. Table 10.4- 5 provides an overview of the results obtained with parent BYI 02960 and its metabolites.

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Table 10.4- 5: Chronic toxicity of BYI 02960 and BYI 02960 metabolites to adult honey bees in the laboratory

Test substance	Ecotoxicological Endpoint: NOEC / NOED		Reference
BYI 02960	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.464 µg a.i./bee/day	[redacted], 2011, M-400539-01-2 KIIA 8.16.1/01
BYI 02960-DFEAF	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.435 µg a.i./bee/day	[redacted], 2012, M-425174-01-2 KIIA 8.16.1/02
BYI 02960-OH	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.420 µg a.i./bee/day	[redacted], 2012, M-425212-01-2 KIIA 8.16.1/03
DFA	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.379 µg a.i./bee/day	[redacted], 2012, M-425105-01-1 KIIA 8.16.1/04
6-CNA	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.418 µg a.i./bee/day	[redacted], 2012, M-425155-01-2 KIIA 8.16.1/05
BYI 02960-CHMP	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.413 µg a.i./bee/day	[redacted], 2012, M-425159-01-2 KIIA 8.16.1/06

Foliage residual toxicity of BYI 02960 to honey bees in the laboratory

There are currently no harmonised and ring tested test guidelines in Europe to assess the residual foliage toxicity to honey bees. Nonetheless, in the USA, there are test guidelines available investigating the toxicity of foliar residues on honey bees under laboratory conditions. As BYI 02960 is applied to the foliage via spray, the SL 200 straight formulation was selected for this study.

Table 10.4- 6: Chronic toxicity of BYI 02960 honey bee larvae in the laboratory

Test substance	Ecotoxicological Endpoint: NOEC / NOED	Reference
BYI 02960 SL 200	No treatment-related adverse effects on behaviour and on survival of honey bees were observed when the bees were exposed for 24 hours to alfalfa foliage, collected after 3, 8 and 24 hours after treatment with BYI 02960 200 SL at a rate corresponding to 205 g a.i./ha.	[redacted] & [redacted] 2011 M-413084-01-1 KIIIA1 10.4.3/01

Chronic toxicity of BYI 02960 to honey bee larvae

There are currently no harmonised and ring tested test guidelines available to assess the chronic risk to honey bee larvae. Nonetheless, there is experience in Europe within some laboratories on conducting chronic studies in honey bee larvae. A study has been performed with the active substance, a detailed summary is included in the Annex II section 6, point 8.16.1, a brief summary of the conclusions is additionally included in this chapter under KIIIA 1 10.4.6/01.

Table 10.4- 7: Chronic toxicity of BYI 02960 honey bee larvae in the laboratory

Test substance	Ecotoxicological Endpoint: NOEC / NOED		Reference
BYI 02960	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./kg larval diet ≥ 0.44 µg a.i./bee larvae/day	[redacted] *et al.*, 2010 M-406645-01-1, KIIIA1 10.4.6.1/01

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Effects of BYI 02960 on honey bees and honey bee colonies under forced exposure conditions

In total, six honey bee semi-field studies under forced exposure conditions have been conducted after foliar applications of BYI 02960 to the highly bee attractive surrogate crop *Phacelia tanacetifolia*. Five of the six semi-field studies complied with the provisions of the EPPO 170 guideline; one study additionally complied with the provisions of the OECD Guidance Document 75 (detailed photographic brood assessment). Out of six honey bee semi-field studies under forced exposure conditions, two studies were non-GLP pilot research studies, four studies were conducted in Germany, one study in Denmark and one study in Italy.

Table 10.4- 8: Effects of on honey bees and honey bee colonies under forced exposure conditions (Pilot studies)

Test substance	Crop / Study Type / Result	Reference
Pilot research tunnel studies (non-GLP), following the provisions of EPPO 170		
BYI 02960 SL 100	*Phacelia tanacetifolia*, semi-field pilot research tunnel study, [redacted] Germany, 2008 The results of the study revealed that BYI 02960 can be applied via foliar application at a rate corresponding to 75 g a.i./ha and 150 g a.i./ha, respectively, into a full-flowering and highly bee attractive crop, as represented here by *Phacelia tanacetifolia*, during honey bees actively foraging on the crop, without adverse effects on mortality, foraging activity, behaviour, brood-, food-, and population development as well as on overall hive vitality.	[redacted], 2012 M-427040-01-1 KIIIA1 10.4.7/01
BYI 02960 SL 200	*Phacelia tanacetifolia*, semi-field pilot research tunnel study, [redacted], Germany, 2009 The results of the study revealed that BYI 02960 can be applied via foliar application at a rate corresponding to 150 g a.i./ha into a full-flowering and highly bee attractive crop, as represented here by *Phacelia tanacetifolia*, during honey bees actively foraging on the crop, without adverse effects on mortality, foraging activity, behaviour, brood-, food-, and population development as well as on overall hive vitality.	[redacted], 2012 M-427046-01-1 KIIIA1 10.4.7/02

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Table 10.4- 9: Effects of on honey bees and honey bee colonies under forced exposure conditions

Test substance	Crop / Study Type / Result	Reference
GLP-compliant tunnel studies, following the provisions of EPPO 170		
BYI 02960 SL 200	<u>*Phacelia tanacetifolia*, semi-field tunnel study, [redacted], Germany, 2009</u> The 1st application of BYI 02960 was a foliar application to the *Phacelia*-crop at BBCH 58, just before onset of flowering, without honey bees present. This 1st application was followed by a 2nd application on the same *Phacelia*-crop during honey bees actively foraging on the full flowering crop at BBCH 65. Both foliar applications corresponded to an application rate of 200 g a.i./ha,. Mortality, flight intensity and behaviour was recorded daily throughout the confinement period, food-, brood- and colony development as well as overall hive vitality was recorded at weekly intervals until 4 weeks after the last application. The outlined BYI 02960 application scenario did not result in treatment-related adverse effects on mortality, foraging activity, behaviour, brood-, food-, and population development or on colony vitality throughout the entire observation time. A slight repellent effect of the test item was observed on the day of the 2nd BYI 02960 application, as well as on the two following days thereafter.	[redacted], 2012 M-425576-01-2 KIIIA1 10.4.7/03
BYI 02960 SL 200	<u>*Phacelia tanacetifolia*, semi-field tunnel study, [redacted], Denmark, 2010</u> The 1st application of BYI 02960 was conducted on bare soil at a rate corresponding to 300 g a.i./ha, followed by immediate soil incorporation and sowing of *Phacelia*-seeds. The 2nd application of BYI 02960 was a foliar application to the *Phacelia*-crop, grown in BYI 02960-treated soil, at BBCH 60, just before onset of flowering, without honey bees present. This 2nd application was followed by a 3rd application on the same *Phacelia*-crop during honey bees actively foraging on the full flowering crop at BBCH 65. Both foliar applications corresponded to an application rate of 200 g a.i./ha, respectively. Mortality, flight intensity and behaviour was recorded daily throughout the confinement period, food-, brood- and colony development as well as overall hive vitality was recorded at weekly intervals until 4 weeks after the last application. The outlined BYI 02960 application scenario has not resulted in treatment-related adverse effects on mortality, foraging activity, behaviour, brood-, food-, and population development as well as on colony vitality throughout the entire observation time. A slight repellent effect of the test item was observed on the day of the 3rd BYI 02960 application.	[redacted], 2012 M-423156-01-2 KIIIA1 10.4.7/04
BYI 02960 SL 200	<u>*Phacelia tanacetifolia*, semi-field tunnel study, [redacted], Italy, 2011</u> The 1st application of BYI 02960 was conducted on bare soil at a rate corresponding to 300 g a.i./ha, followed by immediate soil incorporation and sowing of *Phacelia*-seeds. The 2nd application of BYI 02960 was a foliar application to the *Phacelia*-crop, grown in BYI 02960-treated soil, at BBCH 58-61, just before onset of flowering, without honey bees present. This 2nd application was followed subsequently by a 3rd application on the same *Phacelia*-crop during honey bees actively foraging on the full flowering crop at BBCH 63-68. Both foliar applications corresponded to an application rate of 200 g a.i./ha. Mortality, flight intensity and behaviour was recorded daily throughout the confinement period, food-, brood- and colony development as well as overall hive vitality was recorded at weekly intervals until 4 weeks after the last application. The outlined BYI 02960 application scenario has not resulted in treatment-related adverse effects on mortality, foraging activity, behaviour, brood-, food-, and population development as well as on colony vitality throughout the entire observation time. A slight repellent effect of the test item was observed on the day of the 3rd test item application, after the treatment.	[redacted], 2012 M-423172-01-2 KIIIA1 10.4.7/05

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Table 10.4- 10: Effects of on honey bees and honey bee colonies under forced exposure conditions

Test substance	Crop / Study Type / Result	Reference
GLP-compliant tunnel study with special focus on the development of bee brood in individually marked cells via digital image analysis, following the provisions of the OECD Guidance Document No. 75		
BYI 02960 SL 200	<u>*Phacelia tanacetifolia*, semi-field tunnel study, digital image analysis of individually marked cells, [redacted]), Germany, 2011</u> The 1st application of BYI 02960 was a foliar application to the *Phacelia*-crop at BBCH 59-61, just before onset of flowering, without honey bees present. This 1st application was followed by a 2nd application on the same *Phacelia*-crop during honey bees actively foraging on the full flowering crop at BBCH 63-65. Both foliar applications corresponded to an application rate of 200 g a.i./ha. Mortality, flight intensity and behaviour was recorded daily throughout the confinement period, food-, brood- and colony development as well as overall hive vitality was recorded at regular intervals until 4 weeks after the last application. Particular attention was paid to bee brood development, which quantitatively assessed via digital image analysis of individually marked cells. The outlined BYI 02960 application scenario did not result in treatment-related adverse effects on the survival of marked eggs (brood termination rate), on brood development from eggs into adult bees (brood index) as well as on the brood compensation ability (brood compensation index). Moreover, the outlined application scenario has not resulted in treatment-related adverse effects on mortality, foraging activity, behaviour, brood-, food-, and population development as well as on colony vitality throughout the entire observation time. A slight repellent effect of the test item was indicated by a reduced flight intensity on the day of the 2nd test item application as well as on some further days during the confined exposure period.	[redacted], 2012 M-427438-01-1 KIIA1 10.4.7/06

IIIA1 10.4.1 Hazard Quotients for bees

An indication of hazard (Hazard Quotient or Q_H) can be derived according to the EPPO risk assessment scheme, by calculating the ratio between the application rate (expressed in g or mL/ha) and the lowest laboratory contact and oral LD_{50} (expressed in µg/bee).

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

Q_H values are calculated using data from the studies performed with technical material and the corresponding formulated product. Q_H values higher than 50 indicate the need of higher tiered tests to clarify the actual risk to honey bees.

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IIIA1 10.4.1.1 Oral exposure Q_{HO}

Table 10.4.1.1- 1: Hazard quotients for bees – oral exposure

Crop	Exposure route	LD_{50} [µg a.i./bee]	Application rate [g/ha]	Hazard quotient Q_{HO}	Trigger	Refined risk assessment
BYI 02960 SL 200						
Hops	oral	3.2	150	47	50	No
Lettuce	oral	3.2	125	39	50	No
BYI 02960 (technical)						
Hops	oral	1.2	150	125	50	Yes
Lettuce	oral	1.2	125	104	50	Yes

The hazard quotients for oral exposure, calculated for BYI 02960 technical, slightly exceed the empirical trigger value for higher tier testing ($Q_{HO} > 50$) for both uses. Therefore, a refined risk assessment will be conducted.

IIIA1 10.4.1.2 Contact exposure Q_{HC}

Table 10.4.1.2- 1: Hazard quotients for bees – contact exposure

Crop	Exposure route	LD_{50} [µg a.i./bee]	Application rate [g/ha]	Hazard quotient Q_{HO}	Trigger	Refined risk assessment
BYI 02960 SL 200						
Hops	contact	15.7	150	9.6	50	No
Lettuce	contact	15.7	125	8.0	50	No
BYI 02960 (technical)						
Hops	contact	122.8	150	1.22	50	No
Lettuce	contact	122.8	125	1.02	50	No

The hazard quotient for contact exposure is below the empirical trigger value for higher tier testing ($Q_{HO} > 50$) for both envisaged uses. Therefore, no unacceptable risk to bees is to be expected via the contact route of exposure.

Higher Tier Risk Assessment

The envisaged use of BYI 02960 in hops is an application rate of 150 g a.i./ha, also during the flowering period of the crop. However, hop is a dioecious plant, with male and female flowers developing on separate plants. The harvested commodity in commercial hop cultivation is the female flower cluster. Male hop plants, which could theoretically offer honey bees pollen, are not cultivated in hop plantations – on the contrary: as any pollination of the female flowers (by male pollen) would significantly reduce hop quality, would significantly reduce the harvesting window and would significantly hamper the processing of the hop in breweries, hop plantations are entirely cultivated with female plants. Female flowers, however, do not offer honey bees nectar and pollen. As such, the commercial hop plantations themselves are not attractive to honey bees at any growth stage. Consequently, exposure of honey bees in hop plantations can be expected to be very limited.

The envisaged use of BYI 02960 in lettuce accounts for an application rate of 125 g a.i./ha, during the development of harvestable, vegetative plant parts. Lettuce, however, does not flower in commercial cultivation and as such, honey bees are not attracted by the crop. Moreover, spray applications of BYI 02960 during flowering are excluded by the envisaged application window.

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As such, it can already be concluded at this stage of the risk assessment that due to the very limited exposure situation in hops and lettuce, there is no unacceptable risk for honey bees and honey bee colonies.

Moreover, there are in total six independent semi-field (gauze tunnel) studies, where BYI 02960 formulations were applied to the highly bee attractive surrogate crop *Phacelia tanacetifolia* with honey bees actively foraging on the crop (i.e. during bee flight).
In one pilot research semi-field study, BYI 02960 was applied during bee flight at a rate of 75 and 150 g a.i./ha, respectively, and in a 2nd pilot research semi-field study, BYI 02960 was applied during bee flight at a rate of 150 g a.i./ha.
In four further independent (GLP-compliant) semi-field studies, conducted in Germany, Denmark and Italy, BYI 02960 was always applied at a rate corresponding to 200 g a.i./ha during full flowering of *Phacelia tanacetifolia* with honey bees actively foraging on the crop (i.e. during bee flight). In all of these four GLP-compliant semi-field studies, the *Phacelia*-crop received, in addition to the full-flowering treatment, a pre-flowering application, just before onset of flowering, also at a rate corresponding to 200 g a.i./ha. In two of these four GLP-compliant semi-field studies, there was in addition to the two sequential foliar applications also a soil treatment at a rate corresponding to 300 g a.i./ha at the day of sowing of the *Phacelia*-seeds. In one of these four GLP-compliant semi-field studies, additionally a detailed, quantitative digital brood assessment of individually marked cells has been conducted.

The results of all of the six semi-field (tunnel) studies under forced and as such worst-case exposure conditions consistently did not show any adverse effects on mortality, foraging activity, behaviour, brood-, food- and population development or on overall colony vitality.

The findings as elaborated under forced exposure conditions in gauze tunnels are therefore in line to the findings of the *in-vitro* acute and chronic laboratory studies with BYI 02960, BYI 02960 SL200 and BYI 02960 metabolites. BYI 02960-products are of moderate acute toxicity to honeybees, all plant metabolites of BYI 02960 are virtually non-toxic to honey bees. Moreover, in chronic laboratory feeding studies, where both, parent BYI 02960 and its metabolites have been fed *ad libitum* for a period of 10 consecutive days at a concentration of (up to and including) 10000 µg a.i./L, no effects on mortality have been observed in any of the compounds under investigation.
The lowest $NOED_{oral}$ from acute laboratory toxicity studies with technical BYI 02960 and BYI 02960-products accounts to 0.68 µg a.i./bee, which is well in line to the NOED from the chronic laboratory toxicity study with BYI 02960 of ≥0.46 µg a.i./bee/day. As such, there is no indication of any delayed or particularly chronic effects in honey bees. Moreover, the comparison of the chronic laboratory toxicity studies of BYI 02960 metabolites with the corresponding chronic toxicity study with parent BYI 02960 gives no indication that the BYI 02960 metabolites are of any higher toxicity regarding potentially delayed or chronic effects than the parent compound, which is again in line with the findings of the acute studies.

In addition, parent BYI 02960 was subject to testing in honey bee larvae. The chronic *in-vitro* laboratory study did not reveal adverse effects on honey bee larvae and their development until the imago stage at concentrations of up to and including 10000 µg a.i./kg larval diet. The NOEC of

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10000 µg a.i./kg larval diet translates into a NOED of ≥0.44 µg a.i./bee larvae/day and reveals that there are no indications of a higher toxicity of BYI 02960 to honey bee brood as compared to adult bees (NOED ≥0.46 µg a.i./bee/day). These findings are confirmed by the results of all semi-field studies in general and by the results of the confined semi-field brood study according to the provisions of the OECD Guidance Document No. 75 in particular, where a foliar application of BYI 02960 during full-flowering of the *Phacelia*-crop and during honey bees actively foraging on the crop, did not result in in treatment-related adverse effects on the survival of marked eggs (brood termination rate), on brood development from eggs into adult bees (brood index) as well as on the brood compensation ability (brood compensation index).

Overall, the laboratory database shows that BYI 02960 does not exhibit delayed or chronic effects, either in adult bees or in honey bee larvae. BYI 02960 metabolites are virtually non-toxic to honey bees and there is no indication that BYI 02960 metabolites are of any higher toxicity regarding potentially delayed or chronic effects than the parent compound. These laboratory findings have been consistently confirmed by in total six independent semi-field tunnel studies in the highly bee attractive surrogate crop *Phacelia tanacetifolia*. As such, it can be concluded that BYI 02960 can be applied at foliar application rates of up to and including 200 g a.i./ha, even to bee-attractive, full-flowering crops during honey bees actively foraging, without adverse effects on honey bees, honey bee brood and honey bee colonies.

IIIA1 10.4.2 Acute toxicity of the preparation to bees

IIIA1 10.4.2.1 Acute oral toxicity

Report:	**KIIIA1 10.4.2.1/01; [redacted] (2009)**
Title:	Effects of BYI 02960 SL 200 G (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory
Report No:	52331035
Document No:	M-359920-02-1
Guidelines:	**OECD Guideline 213** **OECD Guideline 214**
Deviations:	**For the contact test, a 5 µL droplet was chosen (for any of the treatments) 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 SL 200 G (Sample description: FAR01438-00 (Batch ID: 2010-00106; Material No.: 79718845; Specification No.: 102000021884-01)) on the honey bee (*Apis mellifera*) after oral or contact exposure.

In the oral dose response test 30 honey bees (adult worker bees) were exposed for 48 hours to doses of 5.6, 4.0, 2.6, 1.3, 0.70 and 0.34 µg a.i. per bee by feeding (value based on the actual intake of the test item). For the contact dose-response test 30 worker bees per treatment were exposed for 72 hours to doses of 200.0, 100.0, 50.0, 25.0, 12.5 and 6.3 µg a.i. per bee by topical application. The contact toxicity test was prolonged for 24 hours due to increasing mortality between 24 and 48 hours, up to a maximum of 72 hours.

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In addition, a negative control (water + 0.5% Adhäsit (contact test); 50% sugar solution (oral test)) and a toxic reference (Dimethoate; 400 g/L nominal) at nominal rates of 0.31, 0.16, 0.08 and 0.06 µg dimethoate/bee, respectively, were tested.

The toxicity of BYI 02960 SL 200 G was tested in both an acute contact and oral toxicity test on honey bees. The LD_{50} (24, 48 + 72 h) of the test item was determined to be 21.5, 17.1 and 15.7 µg a.i./bee in the contact toxicity test, respectively. The LD_{50} (24 h + 48 h) was 3.2 µg a.i./bee in the oral toxicity test.

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, dark brown
Specification number:	102000021884
Batch No.:	2009-001233
Material number:	70718845
Sample description:	FAR01438-00
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.0% w/w, 199.8 g/L, according to certificate of analysis
Solubility:	In water: soluble
Density:	1.175 g/mL (20°C)
Stability of test compound:	Expiry date: 20.03.2010, when stored at 25 ± 5°C in original container in the dark (also acceptable from +2 to +30°C)

Control

Oral Test:	50 % aqueous sugar solution (in tap water)
Contact Test:	Tap water with 0.5% Adhäsit* (applied after anesthetization with CO_2) * (Adhäsit improves spreading of the test droplet on the water-repellent hairs on the thorax of bees)

Wetting Agent

Name:	Adhäsit
Batch No.:	0150207
Analytical content of active ingredient:	100 g/L Marlopon (nominal)
Manufacturer:	[redacted]
Storage:	Expiry Date: 12/2009, when stored in original container, at room temperature (10°C to 30°C), in the dark
Target Amount in this Study:	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:	[redacted]
Batch No.:	FRE-000627
Nominal content of active ingredient:	Dimethoate: 400 g/L
Analytical content of active ingredient:	Dimethoate: 422.4 g/L according to certificate of analysis
Certificate of Analysis Study Code:	346282_14
Type of formulation:	EC
Chemical state and description:	Liquid, blue
Density:	1.076 g/cm^3
Solubility:	In water: emulsifiable
Stability:	Expiry date: 31.10.2009, when stored in original container, in refrigerator (4 ± 4 °C), in the dark

2. Test organisms

Species:	*Apis mellifera carnica* L.
Common name:	Honey bee
Age or developmental stage at test start:	Adult female worker bees
Source:	Honey bee colonies, disease-free and queen-right, bred by IBACON

B. Study design and methods

1. In life dates: August 24 to 27, 2009

2. Experimental treatments:

Test units were stainless steel chambers of 10 cm x 8.5 cm x 9.5 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 walls were lined with filter paper.

10 bees were used per test unit, 3 replicates per test item dose level, controls and toxic standard dosages (i.e. 30 individuals per treatment group).

Exposure time for both tests was 48 hours. However, due to increasing mortality between 24 and 48 hours the contact test was prolonged for a further 24 hours up to 72 hours.

Food was commercial ready-to-use syrup (Apiinvert; 30% Saccharose, 31% Glucose, 39% Fructose).

Control: Contact test: CO_2/tap water + Adhäsit[8] treated control; Oral test: tap water/ syrup control.

Test item:

Contact Test:

Nominal dosage 200, 100, 50, 25, 12.5 and 6.3 µg of BYI 02960 SL 200 G/bee

Oral Test:

Nominal dosage 10, 5, 2.5, 1.3, 0.63 and 0.31 µg of BYI 02960 SL 200 G/bee

Measured dosage 5.6, 4.0, 2.6, 1.3, 0.70 and 0.34 µg of BYI 02960 SL 200 G/bee

Toxic reference item:

[8] Adhäsit was used to improve the spreading of the test droplet on the bee body. Adhäsit is non-toxic to honey bees.

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Contact test:

Nominal dosage 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

Measured dosage 0.31, 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. One single 5 µL droplet of BYI 08330 100OD in solvent (solvent = water + 1% Adhäsit) was placed on the ventral bee thorax using a Burkard - Applicator. For the control one 5 µL droplet of tap water with 1% Adhäsit was used. The toxic standard was applied in 5 µL tap water with 1% Adhäsit (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 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:

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 bees was 50%. For the controls water and sugar syrup was used at the same ratio (1 + 1). The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake ranged from 40 minutes to 6 hours 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 fresh, untreated food. The target dose levels (*e.g.* 10 µg a.i./bee nominal) would have been obtained if 20 mg/bee of the treated food was ingested. In practice, higher or lower 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 4 hours (first day); 24 and 48 hours (contact and oral test), and additionally after 72 hours (contact test). Behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4 hours (first day); 24 and 48 hours (contact and oral test); additionally after 72 hours (contact 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 test item and the toxic standard were estimated with Probit Analysis (according to Finney 1971).

The contact and oral LD_{50} of the reference item were estimated according to moving average computations ([redacted], 1952).

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The LD_{50} calculation was carried out taking into account the mortality data corrected by control mortality using Abbott's formula (1925) [not necessary].

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.

RESULTS AND DISCUSSION

A. Environmental Parameters

Measurements of climatic parameters during the test are summarized as follows:

Test environment:	Incubator
Test temperature:	25°C
Relative air humidity:	61 to 78 %
Light intensity:	Darkness (except during observation)
Ventilation:	Ventilation to avoid possible accumulation of pesticide vapour
Recording:	Test conditions were continuously recorded with electronic data logger and documented in the raw data

B. Biological Findings

Oral Test:

In the oral test, the maximum nominal dose levels of the test item (10 and 5 µg a.i./bee) could not be achieved, because the bees did not ingest the full volume of treated sugar solution even when offered over a period of 6 hours. Oral doses of 5.6, 4.0 and 2.6 µg a.i. per bee resulted in mortality ranging from 93.3% to 16.7% at the end of the test (48 hours after application). No mortality occurred in the 1.3, 0.70 and 0.34 µg a.i./bee dose levels or in the control (50% sugar solution). During the 4 hours assessment movement coordination problems and/or apathy were observed in the three highest dose groups (5.6, 4.0 and 2.6 µg a.i./bee). After 24 hours one bee showed a discoordinated movement in the 5.6 µg a.i./bee group. No behavioural abnormalities occurred in the other dose levels.

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Table 10.4.2.1- 4: Mortality and behavioural abnormalities of the bees in the oral toxicity test

consumed dose µg a.i./bee	after 4 hours mortality mean %	after 4 hours behav. abnorm. mean %	after 24 hours mortality mean %	after 24 hours behav. abnorm. mean %	after 48 hours mortality mean %	after 48 hours behav. abnorm. mean %
test item						
5.6	63.3	36.7	93.3	3.3	93.3	0.0
4.0	73.3	26.7	93.3	0.0	93.3	0.0
2.6	6.7	16.7	16.7	0.0	16.7	0.0
1.3	0.0	0.0	0.0	0.0	0.0	0.0
0.70	0.0	0.0	0.0	0.0	0.0	0.0
0.34	0.0	0.0	0.0	0.0	0.0	0.0
water	0.0	0.0	0.0	0.0	0.0	0.0
reference item						
0.31	93.3	6.7	100.0	0.0	100.0	0.0
0.16	0.0	0.0	86.7	0.0	86.7	0.0
0.08	3.3	0.0	13.3	0.0	13.3	0.0
0.06	0.0	0.0	6.7	0.0	6.7	0.0

results are averages from three replicates (ten bees each) per dosage/control

behav. abnorm. = behavioural abnormalities; water = water control

Contact Test:

The contact test was prolonged up to 72 hours due to increasing mortality between 24 and 48 hours. Mortality occurred in all groups dosed with BYI 02960 SL 200 G ranging from 100.0 to 10.0% at the end of the test (72 hours). No mortality occurred in the control (water + 0.5% Adhäsit). During the entire time of the experiment, a few bees in the higher dose levels (200.0, 100.0, 50.0 and 25.0 µg a.i./bee) were behaving abnormally (*e.g.* movement coordination problems and/or intensive cleaning). No behavioural impairments occurred in the 12.5 and 6.3 µg a.i./bee dose groups at any time.

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Table10.4.2.1- 3: Mortality and behavioural abnormalities of the bees in the contact toxicity test

dosage µg a.i./bee	after 4 hours mortality mean %	after 4 hours behav. abnorm. mean %	after 24 hours mortality mean %	after 24 hours behav. abnorm. mean %	after 48 hours mortality mean %	after 48 hours behav. abnorm. mean %	after 72 hours mortality mean %	after 72 hours behav. abnorm. mean %
test item								
200.0	96.7	3.3	100.0	0.0	100.0	0.0	100.0	0.0
100.0	50.0	26.7	100.0	0.0	100.0	0.0	100.0	0.0
50.0	46.7	40.0	80.0	6.7	90.0	3.3	93.3	3.3
25.0	10.0	10.0	63.3	16.7	76.7	0.0	76.7	3.3
12.5	0.0	0.0	30.0	0.0	36.7	0.0	36.7	0.0
6.3	0.0	0.0	0.0	0.0	3.3	0.0	10.0	0.0
water	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
reference item								
0.30	3.3	10.0	83.3	10.0	96.7	0.0	96.7	0.0
0.20	6.7	3.3	60.0	0.0	66.7	0.0	66.7	0.0
0.15	0.0	0.0	16.7	10.0	46.7	0.0	56.7	6.7
0.10	0.0	0.0	0.0	3.3	20.0	3.3	26.7	0.0

results are averages from three replicates (ten bees each) per dosage/control
behav. abnorm. = behavioural abnormalities;
water = CO_2/water-treated 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.

D. Biological Endpoints Derived

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

Table 10.4.2.1 - 1: Toxicity to Honey Bees: laboratory tests

Acute contact toxicity test:	Contact $LD_{50\ (24h)}$ of BYI 02960 SL 200 G: 21.5 µg a.i./bee Contact $LD_{50\ (48h)}$ of BYI 02960 SL 200 G: 17.1 µg a.i./bee Contact $LD_{50\ (72h)}$ of BYI 02960 SL 200 G: 15.7 µg a.i./bee Contact $NOED_{(72h)}$ of BYI 02960 SL 200 G: 6.3 µg a.i./bee
Acute oral toxicity test:	Oral $LD_{50\ (24h)}$ of BYI 02960 SL 200 G: 3.2 µg a.i./bee Oral $LD_{50\ (48h)}$ of BYI 02960 SL 200 G: 3.2 µg a.i./bee Oral $NOED_{(48h)}$ of BYI 02960 SL 200 G: 2.6 µg a.i./bee
Behavioural Abnormalities:	Behavioural abnormalities such as moving coordination problems, apathy and/or intensive cleaning occurred in the contact and oral toxicity test.

Contact toxicity test:

Table 10.4.2.1- 2: Test item contact LD_{50}

Test Item Contact LD_{50}:	24 h	48 h	72 h
	21.5 µg a.i./bee	17.1 µg a.i./bee	15.7 µg a.i./bee
95 %- Confidence limit (lower):	17.6 µg a.i./bee	14.0 µg a.i./bee	12.7 µg a.i./bee
95 %- Confidence limit (upper):	26.0 µg a.i./bee	20.6 µg a.i./bee	19.0 µg a.i./bee

Oral toxicity test:

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Table 10.4.2.1- 3: Test item oral LD_{50}

Test Item Oral LD_{50}:	24 h	48 h
	3.2 µg a.i./bee	3.2 µg a.i./bee
95 %- Confidence limit (lower):	2.3 µg a.i./bee	2.3 µg a.i./bee
95 %- Confidence limit (upper):	3.9 µg a.i./bee	3.9 µg a.i./bee

CONCLUSION

The toxicity of BYI 02960 SL 200 G was tested in both an acute contact and oral toxicity test on honey bees. The LD_{50} (24, 48 + 72 h) of the test item was determined to be 21.5, 17.1 and 15.7 µg a.i./bee in the contact toxicity test, respectively. The LD_{50} (24 h + 48 h) was 3.2 and 3.2 µg a.i./bee in the oral toxicity test, respectively. Behavioural abnormalities such as moving coordination problems, apathy and/or intensive cleaning occurred in both the contact and oral toxicity test.

IIIA1 10.4.2.2 Acute contact toxicity

The acute contact toxicity study on honeybees are summarized in Point IIIA1 10.4.2.1 above.

IIIA1 10.4.3 Effects on bees of residues on crops

Report:	**KIIIA1 10.4.3/01; [redacted] J.R., [redacted] H.O., (2011)**
Title:	BYI 02960 200 SL: A foliage residue toxicity study with the honeybee
Report No:	EBRVP044
Document No:	M-413084-01-1
Guidelines:	**OPPTS No. 850.3030** **FIFRA Subdivision L, Section 141-2**
Deviations:	**The concentration, homogeneity and stability of the test substance in the carrier (foliage) were not determined.**
GLP	**Yes (certified laboratory)** **Periodic analyses of well water for potential contaminants were not conducted in accordance with Good Laboratory Practice Standards; however, these analyses were performed using a certified laboratory and standard U.S. EPA analytical methods.**

Executive summary

The objective of this study was to evaluate the toxicity to the honeybee (*Apis mellifera*) of residues of BYI 02960 200 SL (Batch ID: 2010-001187, Specification No.: 102000021884) on plant foliage after weathering for various time periods. Mortality of the bees and sublethal effects such as changes in behavior were evaluated.

Honeybees were exposed to three treatment groups and one control group. Bees in each treatment group were exposed for 24 hours to alfalfa foliage sprayed with an aqueous mixture of the test substance at a nominal application rate of 205 g a.i./ha or 1.025 L product/ha (14 oz product/ac). This application rate represented the single maximum label use rate for the BYI 02960 200 SL formulation. The three treatment groups differed from one another in the length of time that residues were allowed to age on the foliage prior to harvest. Groups of plants were sprayed 3, 8 and 24 hours prior to collection of the foliage, with a control group sprayed with well water purified by reverse osmosis at the shortest aging interval (3 hours).

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In conclusion, honeybees showed no treatment-related effects on behavior or survival when exposed for 24 hours to alfalfa foliage collected at 3, 8 and 24 hours after application of BYI 02960 200 SL at the maximum label rate of 1.025 L product/ha (205 g a.i./ha).

MATERIAL AND METHODS

A. Materials

1. Test material

Test item:	BYI 02960 SL 200 G
Specification number:	102000021884
Type:	Formulated product (soluble liquid concentrate)
Chemical state and description:	Clear brown liquid
Batch No.:	2010-001187
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.11% according to certificate of analysis
Density:	1.175 g/mL (20°C)
Stability of test compound:	Expiry date: 14.01.2013, when stored from +2 to +30°C)
Control:	Well water purified by reverse osmosis, sprayed at shortest aging interval (3 hours pre-harvest)

2. Test organisms

Species:	*Apis mellifera*
Common name:	Honey bee
Age or developmental stage at test start:	Young adult worker bees
Source:	[redacted]
Plant species used for the test:	Alfalfa (*Medicago sativa*)

B. Study design and methods

1. In life dates: May 23 to 25, 2011

2. Experimental treatments:

Honeybees were exposed to three treatment groups and one control group. Bees in each treatment group were exposed for 24 hours to alfalfa foliage sprayed with an aqueous mixture of the test substance at a nominal application rate of 205 g a.i./ha or 1.025 L product/ha. This application rate represented the maximum label use rate for this BYI 02960 200 SL formulation. The three treatment groups differed from one another in the length of time that residues were allowed to age on the foliage prior to harvest. Groups of plants were sprayed 3, 8 and 24 hours prior to collection of the foliage, with a control group sprayed with well water purified by reverse osmosis at the shortest aging interval (3 hours).

Alfalfa foliage was harvested from treatment and control plants after aging for the appropriate time. Equal aliquots of foliage were placed in each of six replicate test chambers assigned to each treatment and control group and 25 worker honeybees were added to each test chamber. The bees were exposed to the foliage for approximately 24 hours.

The experimental design, spray application and harvest intervals are summarized in the table below.

Experimental Design, Application and Harvest Times					
Residue Aging Interval [1]	Date and Time of Application	Date and Time of Harvest	No. of Replicates	No. Bees Per Test Chamber	Total No. Bees Per Treatment
Control	May 24, 2011 10:57	May 24, 2011 1400	6	25	150
24 Hours	May 23, 2011 13:56	May 24, 2011 1404	6	25	150
8 Hours	May 24, 2011 06:00	May 24, 2011 1409	6	25	150
3 Hours	May 24, 2011 11:00	May 24, 2011 1413	6	25	150

[1] The number of hours from time of application until the harvest of the treated foliage.

Bees were collected on the day of the test and delivered to the laboratory in a screened box. After their introduction into the test chambers the bees were provided 50% sucrose solution (prepared w/v with deionized water). The test chambers were ventilated stainless steel cylinders.

3. Observation and measurements:

The bees were observed for mortality and signs of toxicity following introduction to the foliage in the test chambers. The bees were observed once within the first four hours after initiation of exposure and again at approximately 24 hours after initial exposure. During the first observation period, the bees were observed without removing them from the test chambers. Therefore, only an estimation of mortality and effects was possible from those bees visible on the foliage. At the final observation period, the bees and foliage were removed from the test chambers to obtain an accurate assessment of mortality.

The mean percent mortality of the honeybees exposed to foliage for each residue-aging interval for 24 hours was determined. No statistical methods were employed for analyzing mortality data; results were reported as percentages.

RESULTS AND DISCUSSION

A. Environmental parameters

The environmental conditions recorded during aging of the sprayed foliage included light intensity, temperature, and relative humidity. Due to the possibility of rainfall during the night of the 24-hour residue-aging period, treated plants were held overnight in a greenhouse to prevent the residues from being washed off of the foliage.

During the exposure phase of the test (24 hours), bees were maintained in an environmental chamber at a temperature of 26 to 27°C, with a relative humidity of 51-55%.

B. Biological Findings

Data from observations of the bees for mortality and other signs of toxicity during the approximately 24-hour exposure are shown in the following table.

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Table 10.4.3- 1: Cumulative Mortality and Observations of Honeybees Exposed to Alfalfa Foliage Treated with BYI 02960 200 SL

Treatment Group (length of time residues aged on foliage)	Replicate	~1 Hour of Exposure [1]		24 Hours of Exposure		Replicate Mortality (%)	Group Mortality (%)
		Mortality [2]	Effects [3]	Mortality	Effects		
Control Group	A	0	all AN	0	25 AN	0	2.7
	B	0	all AN	1	24 AN	4	
	C	0	all AN	0	25 AN	0	
	D	0	all AN	0	25 AN	0	
	E	0	all AN	0	25 AN	0	
	F	0	all AN	3	22 AN	12	
24 Hours	A	0	all AN	0	25 AN	0	2.0
	B	1	rest AN	1	24 AN	4	
	C	0	all AN	1	24 AN	4	
	D	0	all AN	0	25 AN	0	
	E	0	all AN	0	25 AN	0	
	F	0	all AN	0	1 I, 24 AN	4	
8 Hours	A	0	all AN	1	24 AN	4	6.0
	B	0	all AN	0	25 AN	0	
	C	0	all AN	3	22 AN	12	
	D	0	all AN	1	24 AN	4	
	E	0	all AN	4	21 AN	16	
	F	0	all AN	0	25 AN	0	
3 Hours	A	0	all AN	0	25 AN	0	2.7
	B	0	all AN	1	24 AN	4	
	C	0	all AN	1	24 AN	4	
	D	0	all AN	0	1 I, 24 AN	4	
	E	0	all AN	0	25 AN	0	
	F	0	all AN	1	24 AN	4	

[1] Observation times represent the approximate number of hours after the start of exposure. The observations conducted at ~1 hour were an estimation based on those bees visible on the foliage. An accurate assessment was made at 24 hours when bees and foliage were removed from the test chambers.
[2] Mortality data are presented as the cumulative number dead or immobile of 25 bees originally exposed per replicate.
[3] Number of bees exhibiting clinical signs: AN = appear normal, I = immobile.

After 24 hours of exposure to treated foliage, immobility/mortality of bees in the control group was 2.7% (4 of 150). All surviving control bees appeared normal. The immobility/mortality of bees exposed to alfalfa foliage treated at the maximum label rate with BYI 02960 200 SL and aged for 3, 8 24 hours and the control was 2.7% (4 of 150), 6.0% (9 of 150) 2.0% (3 of 150) and 2.7 % (4 of 150), respectively. The low level of mortality that occurred in the test was considered to be incidental and not related to treated foliage. After 24 hours of exposure to the treated foliage there were no sublethal treatment-related effects noted in the bees in the treatment groups.

CONCLUSION

Honeybees showed no treatment-related effects on behavior or survival when exposed for 24 hours to alfalfa foliage collected at 3, 8 and 24 hours after application of BYI 02960 200 SL at the maximum label rate of 1.025 L product/ha (205 g a.i./ha).

IIIA1 10.4.4 Cage tests

A series of tunnel studies have been conducted under confined semi-field conditions, these studies are filed under IIIA1 10.4.7.

IIIA1 10.4.5 Field tests

In view of the findings under forced exposure conditions (see IIIA1 10.4.7), field studies are not necessary.

IIIA1 10.4.6 Investigation of special effects

IIIA1 10.4.6.1 Larval toxicity

This study performed with the active substance is fully summarized in the Annex II Tier 2 summary Point 6, Section 8 under KIIA 8.16.1/07, therefore only the summary is included in this Tier 2 Annex III as the study is used in the risk assessment.

Report:	**KIIIA1 10.4.6.1/01; [redacted] A., [redacted] M., [redacted] D.; (2010)**
Title:	BYI 02960 tech.: Effects of exposure to spiked diet on honey bee (*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 de la Recherche Agronomique) - method for testing pesticide toxicity to honeybee brood in laboratory conditions (January, 2008) and the recommendations of the honeybee larvae laboratory ring-test group, organized by ICPBR ([redacted] et al., 2009)**
Deviations:	**Not applicable**
GLP:	**Yes (certified laboratory)** **The rearing of bee larvae in the bee hives was not part of GLP. The preparation of saturated solutions of K_2SO_4 and NaCl and the preparation of solutions for the disinfection of grafting cells as well as for the wetting of dental rolls were not part of GLP. The procedure of the disinfection of grafting cells and the preparation of the rearing plates, respectively test plates were not part of the GLP.**

The full summary for this study is included in the Annex II point 8.16.2.

Executive Summary

The purpose of the biological part of this study was to assess the effects of BYI 02960 tech. (TOX 08508-00; Specification No.: 102000022313; Batch code: BYI 02960-01-03; content of a.i. (analysed): 96.2% w/w) on honey bee larvae, *Apis mellifera carnica*, after artificial feeding of spiked diet in an in 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.

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At day +1 (day 0 was the anticipated day of larval hatching), 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 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 µg 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 ± 5% and from day +8 to +22 the mean relative humidity was about 80 ± 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 self-set validity criteria.

Overall, it can be concluded that the No Observed Effect Concentration (NOEC) as determined in this *in vitro* honeybee larvae study is ≥ 10000 µg BYI 02960 a.i./kg diet.

IIIA1 10.4.6.2 Long residual effects

For the active ingredient and its metabolites BYI 02960-DFEAF, BYI 02960-OH, DFA, 6-CNA and BYI 02960-CHMP, chronic continuous laboratory feeding studies in adult honeybees have been conducted. These are filed in the Annex II document (refer to point IIA 8.16.1).

IIIA1 10.4.6.3 Disorienting effects on bees

No specific study has been conducted in the absence of validated test protocols. However, potential effect on honey bee behaviour on the crop and around the hive have been assessed in a series of tunnel studies, filed under IIIA1 10.4.7.

IIIA1 10.4.7 Tunnel tests - effects of feeding on contaminated honey dew or flowers

Report:	**KIIIA1 10.4.7/01; [redacted] H.-J. (2012)**
Title:	Evaluation of the effects of BYI 02960 SL 100 on honey bees (*Apis mellifera)* in a semi-field tunnel test in full-flowering *Phacelia tanacetifolia*
Report No:	IA08DVG048G001
Document No:	M-427040-01-1
Guidelines:	**OEPP/EPPO Guideline No. 170 (3), 2001**
Deviations:	**None**
GLP:	**No**

Executive summary

The purpose of this study was to examine the effects on honey bees of the insecticide BYI 02960, applied as BYI 02960 SL 100 (nominal content of a.i. BYI 02960, 100 g a.i./L; Batch ID: 2008-000707; Spec.No.: 102000019065) in combination with 20% of the surfactant TANEMUL KS (alkoxylated castor oil), via foliar application under confined conditions, at a rate corresponding to 75 g and 150 g BYI 02960 a.i./ha, respectively, to full-flowering Phacelia tanacetifolia, by evaluating effects on mortality, foraging activity, behaviour, colony strength, brood- and food development and overall hive vitality.

Applications in all experimental groups were performed by applying 400 L spray solution or water/ha to full-flowering Phacelia tanacetifolia under confined conditions by using a calibrated boom sprayer. Small honey bee colonies (with approx. 2500 honey bees) were confined in tunnels (50 m²) on Phacelia tanacetifolia. The test fields were located within the local subdistrict "[redacted]", close to [redacted], North-Rhine Westphalia, Germany.

Three replicates (= three gauze tunnels) were set up for each experimental group, consisting of an untreated control (tap water), the toxic reference Insegar (Insegar® WG 25, nominal content of a.i. fenoxycarb: 25% w/w, employed application rate in the test: 0.6 kg product/ha, corresponding to 150 g fenoxycarb a.i./ha) and two concentrations of the test item (75 g a.i./ha, 150 g a.i./ha), respectively.
In addition, two replicates (=two gauze tunnels) were set up for the toxic reference Actara (Actara® 25 WG, nominal content of the a.i. thiamethoxam: 25% w/w, employed application rate in the test: 0.2 kg product/ha, corresponding to 50 g thiamethoxam a.i./ha).

The honey bees were placed inside the tunnels 4 days before the respective application (DAA-4[9]), in the morning. The small honey bee colonies in all experimental groups were examined daily during the confined exposure period for effects on mortality, behaviour and foraging activity (10 days in succession, from DAA-3 until DAA6). Honey bee colonies were relocated to a monitoring site on DAA8, where the bees were allowed to forage freely. Colony assessments including an assessment of colony strength, eggs, larvae, pupae, nectar- and pollen stores as well as hive weight were performed in regular intervals during and after the confined exposure period until DAA27 (last colony assessment).

All endpoints were compared between control, toxic references and test item before and after the respective application.

The results of the study revealed that BYI 02960 can be applied via foliar application at a rate corresponding to 75 g a.i./ha and 150 g a.i./ha, respectively, into a full-flowering and highly bee attractive crop, as represented here by *Phacelia tanacetifolia*, during honey bees actively foraging on the crop, without adverse effects on mortality, foraging activity, behaviour, brood-, food-, and population development as well as on overall hive vitality.

[9] DAA = Day After Application
a.i. = active ingredient

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MATERIAL AND METHODS

A. Material

1. Test material

Test item:	BYI 02960 SL 100 G
Specification number:	102000019065
Type:	Formulated product (suspension liquid (SL); soluble concentrate)
Chemical state and description:	Liquid (colour not stated)
Batch No.:	2008-000707
Master recipe ID:	0094913-001
Sample code:	08003504
Sample No.:	A.08000052
Nominal content of active ingredient:	BYI 02960: 100 g/L
Analytical content of active ingredient:	BYI 02960: 8.7% w/w, 100 g/L according to certificate of analysis
Density:	1.152 g/mL at 20 °C
Stability of test compound:	Expiry date: 05.08.2008
Surfactant:	TANEMUL KS (20%) (alkoxylated castor oil)
Control:	Untreated water control (400 L/ha tap water)
Reference Item 1:	
Name:	Insegar (WG 25)
Manufacturer:	Syngenta Crop Protection
Batch No.:	Not stated
Nominal content of active ingredient:	Fenoxycarb: 25% w/w
Type of formulation:	WG (water-dispersable granule)
Chemical state and description:	Grey-brown granules
Stability:	To be stored dry, cool and well ventilated
Reference Item 2:	
Name:	Actara (25 WG)
Manufacturer:	Syngenta Crop Protection
Batch No.:	Not stated
Nominal content of active ingredient:	Thiamethoxam: 25% w/w
Type of formulation:	WG (water-dispersable granule)
Chemical state and description:	Beige brown granules
Stability:	To be stored dry, cool and well ventilated

2. Test organisms

Species:	*Apis mellifera carnica* L.
Common name:	Honey bee
Source:	[redacted]. Small, healthy honey bee colonies with three combs from one breeding line. Colony size: approx. 2500 bees / colony at set-up

B. Study design and methods

1. In life dates: June 21 to July 23, 2008

2. Experimental treatments

Location: The study was conducted on test fields that were located within the local subdistrict "[redacted]

Crop: The overall size of the test field was approximately 1000 m² (100 m x 10 m).

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The crop *Phacelia tanacetifolia* was drilled on 21 April 2008 by using a typical and appropriate sowing machine. The employed *Phacelia*-seeds were not seed-treated and the crop has not received any maintenance treatment with plant protection products.

Test unit: In order to prevent honey bees from leaving the test plots and to escape the treatment or to collect nectar or pollen from other sources than the treated crop on the test plots, gauze tunnels, serving as test units, were placed on the respective study plots some days prior to application. The tunnels were covered with gauze, preventing the bee from escaping but allowing for sufficient ventilation (mesh-size approx. 1.5 mm). The floor space of each tunnel was approximately 50 m^2 (5 m width x 10 m length). The distance between the tunnels was approx. 2 - 5 m.

For each experimental group (control, test item and toxic reference), two or three tunnels were set up in the field. The test units (tunnels) were labelled with the study number and all necessary additional information to assure unmistakable identification.

Treatment design:

Number and name of the experimental groups:

1. Untreated (Control; tap water)
2. Insegar (WG 25; a.i. fenoxycarb)
3. BYI 02960 75 g a.i./ha (SL 100; in combination with 20% of the surfactant TANEMUL KS)
4. BYI 02960 150 g a.i./ha (SL 100; in combination with 20% of the surfactant TANEMUL KS)
5. Actara (25 WG; a.i. thiamethoxam)

Number of replicates in the experimental groups 1, 2, 3 and 4:	3
Number of replicates in the experimental group 5:	2
Definition of test unit:	1 test unit = 1 gauze tunnel
Number of bee colonies per test unit:	1
Identification of the test units:	Study number, experimental group / name and replicate of the test unit

3. Observation and measurements:

The purpose of this study was to examine the effects on honey bees of the insecticide BYI 02960, applied in combination with 20% of the surfactant TANEMUL KS (alkoxylated castor oil), via foliar application under confined conditions, at a rate corresponding to 75 g and 150 g BYI 02960 a.i./ha to full-flowering *Phacelia tanacetifolia*, by evaluating effects on mortality, foraging activity, behaviour, colony strength, brood- and food development and overall hive vitality.

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RESULTS AND DISCUSSION

A. Biological Findings

An overview of the findings for foraging activity and mortality for all treatment groups is presented in the below in Table 10.4.7- 1.

Foraging activity after application was comparable in the control group in the test item group (a.i. BYI 02960, at 75 g a.i./ha and 150 g a.i./ha, respectively) as well as in the Insegar (a.i. fenoxycarb) group, while foraging activity decreased strongly to very low levels in the Actara (a.i. thiamethoxam) group. Foraging activity in the Actara (a.i. thiamethoxam) group did not recover during the entire confinement period. In the test item group at 150 g a.i./ha, foraging activity was slightly reduced immediately after the treatment when compared to control, but recovered fully within a couple of hours after treatment and followed the control group at any point in time thereafter.

While worker bee mortality after application was comparably low in the control, in the test item (a.i. BYI 02960 75 g a.i./ha and 150 g a.i./ha) and in the Insegar (a.i. fenoxycarb) group at any point in time during the study, worker bee mortality was strongly increased in the Actara (a.i. thiamethoxam) group. Mortality of worker bees in the Actara (a.i. thiamethoxam) group remained on an elevated level compared to control at least until the end of the confinement period (DAA6).

In none of the experimental groups there was any conspicuous drone mortality.

Except for the Insegar group (a.i. fenoxycarb), where the number of dead pupae was considerably increased from DAA11 until at least DAA14 (potentially from about DAA7 until DAA18), only some single dead pupae were found in any of the other experimental groups.

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Table 10.4.7- 1: Findings

Time Interval	Untreated	Insegar	BYI 02960 (75 g a.i./ha)	BYI 02960 (150 g a.i./ha)	Actara
Foraging activity (Daily average number of flower visits on 1 m² per experimental group)					
	three replicates				two replicates
DAA-3 – DAA-1	29.4	28.7	29.6	29.2	28.7
DAA0ba	24.3	26.2	25.5	28.3	26.5
DAA0aa	40.2	40.8	39.6	37.2	0.4
DAA1	22.8	23.3	22.4	22.8	0.3
DAA2 – DAA3	39.3	35.5	36.8	37.5	0.3
DAA4 – DAA6	39.1	38.1	37.8	37.3	6.7
Mortality (Daily average number of dead worker bees, drones and pupae in front of the hive per group)					
	three replicates				two replicates
Worker bees, DAA-3 – DAA-1	1.4	2.1	0.7	1.3	1.3
Worker bees, DAA0ba	1.7	2.0	1.7	2.3	0.5
Worker bees, DAA0aa	1.3	0.0	0.7	1.7	465.5
Worker bees, DAA1	0.0	1.3	0.0	0.0	153.0
Worker bees, DAA2 – DAA3	0.3	0.3	0.3	0.0	139.8
Worker bees, DAA4 – DAA6	1.9	2.1	2.8	1.4	301.3
Worker bees, DAA7 – DAA11	0.0	0.2	0.0	0.3	3.3
Worker bees, DAA12 – DAA25	0.1	0.3	0.5	0.4	0.4
Drones, DAA-3 – DAA0ba	0.0	0.0	0.0	0.0	0.0
Drones, DAA0aa – DAA25	0.0	0.0	0.0	0.0	0.0
Pupae, DAA-3 – DAA-1	0.1	0.0	0.0	0.0	0.0
Pupae, DAA0ba	0.0	0.0	0.0	0.0	0.0
Pupae, DAA0aa	0.0	0.0	0.0	0.0	0.0
Pupae, DAA1	0.0	0.0	0.0	0.0	0.0
Pupae, DAA2 – DAA3	0.3	0.0	0.2	0.0	0.0
Pupae, DAA4 – DAA6	0.0	0.0	0.0	0.0	0.2
Pupae, DAA7 – DAA11	0.0	1.8	0.1	0.0	0.0
Pupae, DAA12 – DAA25	0.0	3.2	0.0	0.0	0.1

DAA = Day after application; DAA0ba = Day of application, before application; DAA0aa = Day of application, after application

In the Actara (a.i. thiamethoxam) group, the treatment resulted at least in transient effects on hive weight development, the extent of nectar- and pollen stores, on larval- and pupal abundance as well as on population development. In the Insegar (a.i. fenoxycarb) group, the treatment resulted at least in transient effects on larval- and pupal abundance as well as on population development.

No distinct differences were found when comparing the extent of nectar- and pollen stores, egg laying activity, larval and pupal abundance, colony strength, hive weight development and overall hive vitality in the test item treatment groups with control performance at any point in time after application.

C. Validity criteria

The test was considered valid because a detectable effect of the reference item (especially Actara) was found.

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CONCLUSION

The results of the study revealed that BYI 02960 can be applied via foliar application at a rate corresponding to 75 g a.i./ha and 150 g a.i./ha, respectively, into a full-flowering and highly bee attractive crop, as represented here by *Phacelia tanacetifolia*, during honey bees actively foraging on the crop, without adverse effects on mortality, foraging activity, behaviour, brood-, food-, and population development as well as on overall hive vitality.

Report:	**KIIIA1 10.4.7/02; [redacted] H.-J. (2012)**
Title:	Evaluation of the effects of BYI 02960 SL 200 on honey bees (*Apis mellifera)* in a semi-field tunnel test in full-flowering *Phacelia tanacetifolia*
Report No:	IA09DVG051K619
Document No:	M-427046-01-1
Guidelines:	**OEPP/EPPO Guideline No. 170 (3), 2001**
Deviations:	**None**
GLP:	**Non-GLP**

Executive summary

The purpose of this study was to examine the effects on honey bees of the insecticide BYI 02960, applied as BYI 02960 SL 200 (nominal content of a.i. BYI 02960, 200 g a.i./L; Batch No.: 2009-001162; Spec.No.: 102000021884-01) in combination with 40% of the surfactant Antarox B/848 (ethopropoxylated alcohol), via foliar application under confined conditions, at a rate corresponding to 150 g BYI 02960 a.i./ha, respectively, to full-flowering *Phacelia tanacetifolia*, by evaluating effects on mortality, foraging activity, behaviour, colony strength, brood- and food development and overall hive vitality.

Applications in all experimental groups were performed by applying 400 L spray solution or water/ha to full-flowering *Phacelia tanacetifolia* under confined conditions by using a calibrated boom sprayer. Small honey bee colonies (with approx. 2500 honey bees) were confined in tunnels (50 m²) on *Phacelia tanacetifolia* on the premises of Bayer CropScience AG's Experimental Station [redacted], Germany.

Two replicates (= two gauze tunnels) were set up for each experimental group, consisting of an untreated control (tap water), the toxic reference Insegar (Insegar® WG 25, nominal content of a.i. fenoxycarb: 25% w/w, employed application rate in the test: 0.6 kg product/ha, corresponding to 150 g fenoxycarb a.i./ha), the toxic reference Actara (Actara® 25 WG, nominal content of the a.i. thiamethoxam: 25% w/w, employed application rate in the test: 0.2 kg product/ha, corresponding to 50 g thiamethoxam a.i./ha), and one concentrations of the test item (150 g a.i./ha), respectively.

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The honey bees were placed inside the tunnels 2 days before the respective application (DAA-2[10]), in the evening, after bee flight. The small honey bee colonies in all experimental groups were examined daily during the confined exposure period for effects on mortality, behaviour and foraging activity (14 days in succession, from DAA-1 until DAA12). Honey bee colonies were relocated to a monitoring site on DAA13, where the bees were allowed to forage freely. Colony assessments including an assessment of colony strength, eggs, larvae, pupae, nectar- and pollen stores as well as hive weight were performed in regular intervals during and after the confined exposure period until DAA28 (last colony assessment).

All endpoints were compared between control, toxic references and test item before and after the respective application.

The results of the study revealed that BYI 02960 can be applied via foliar application at a rate corresponding to 150 g a.i./ha into a full-flowering and highly bee attractive crop, as represented here by *Phacelia tanacetifolia*, during honey bees actively foraging on the crop, without adverse effects on mortality, foraging activity, behaviour, brood-, food-, and population development as well as on overall hive vitality.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	BYI 02960 SL 200 G
Specification number:	102000021884-01
Type:	Formulated product (suspension liquid (SL), soluble concentrate)
Chemical state and description:	Liquid (colour not stated)
Batch No.:	2009-000162
Master recipe ID:	0102142-001
Sample code:	09035046
Sample No.:	HA.09002458
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.2% w/w, 201.9 g/L according to certificate of analysis
Density:	1.174 g/mL at 20 °C
Stability of test compound:	Expiry date: 22.04.2011
Surfactant:	Antarox B/848 (40%) (ethopropoxylated alcohol)
Control:	Untreated water control (400 L/ha tap water)

Reference Item 1:	
Name:	Insegar (WG 25)
Manufacturer:	Syngenta Crop Protection
Batch No.:	Not stated
Nominal content of active ingredient:	Fenoxycarb: 25% w/w
Type of formulation:	WG (water-dispersable granule)
Chemical state and description:	Grey-brown granules
Stability:	To be stored dry, cool and well ventilated

[10] DAA = Day After Application

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Reference Item 2:

Name:	Actara (25 WG)
Manufacturer:	Syngenta Crop Protection
Batch No.:	Not stated
Nominal content of active ingredient:	Thiamethoxam: 25% w/w
Type of formulation:	WG (water-dispersable granule)
Chemical state and description:	Beige brown granules
Stability:	To be stored dry, cool and well ventilated

2. Test organisms

Species:	*Apis mellifera carnica* L.
Common name:	Honey bee
Source:	[redacted]. Small, healthy honey bee colonies with three combs from one breeding line. Colony size: approx. 2500 - 3000 bees / colony at set-up

B. Study design and methods

1. In life dates: July 07 to August 06, 2009

2. Experimental treatments

Location: The study was conducted on the premises of Bayer CropScience AG's [redacted]

Crop: The overall size of the test field was approximately 1000 m² (100 m x 10 m).

The crop *Phacelia tanacetifolia* was drilled on 30 May 2009 at a sowing rate of 6 kg seeds/ha, by using a typical and appropriate sowing machine (Amazone AP300). The employed *Phacelia*-seeds were not seed-treated and the crop did not receive any maintenance treatment with plant protection products.

Test unit: In order to prevent honey bees from leaving the test plots and to escape the treatment or to collect nectar or pollen from other sources than the treated crop on the test plots, gauze tunnels, serving as test units, were placed on the respective study plots some days prior to application. The tunnels were covered with gauze, preventing the bee from escaping but allowing for sufficient ventilation (mesh-size approx. 1.5 mm). The floor space of each tunnel was approximately 50 m^2 (5 m width x 10 m length). The distance between the tunnels was approx. 3 - 5 m.

For each experimental group (control, test item and toxic references), two tunnels were set up in the field. The test units (tunnels) were labelled with the study number and all necessary additional information to assure unmistakable identification.

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Treatment design:
Number and name of the experimental groups:

1. Untreated (Control; tap water)
2. Insegar (WG 25; a.i. fenoxycarb)
3. BYI 02960, 150 g a.i./ha (SL 200; in combination with 40% of the surfactant Antarox B/848)
4. Actara (25 WG; a.i. thiamethoxam)

Number of replicates per experimental group:	2
Definition of test unit:	1 test unit = 1 gauze tunnel
Number of bee colonies per test unit:	1
Identification of the test units:	Study number, experimental group / name and replicate of the test unit

3. Observation and measurements

The purpose of this study was to examine the effects on honey bees of the insecticide BYI 02960, applied as BYI 02960 SL 200 (nominal content of a.i. BYI 02960, 200 g a.i./L; Batch No.: 2009-001162; Spec.No.: 102000021884-01) in combination with 40% of the surfactant Antarox B/848 (ethopropoxylated alcohol), via foliar application under combined conditions, at a rate corresponding to 150 g BYI 02960 a.i./ha, respectively, to full-flowering *Phacelia tanacetifolia*, by evaluating effects on mortality, foraging activity, behaviour, colony strength, brood- and food development and overall hive vitality.

RESULTS AND DISCUSSION

A. Biological Findings

An overview of the findings for foraging activity and mortality for all treatment groups is presented in the following table.

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Table 10.4.7- 2: Findings

Time Interval	Untreated	Insegar	BYI 02960	Actara
Foraging activity (Daily average number of flower visits on 1 m² per experimental group)				
DAA-1	10.6	12.1	11.9	12.8
DAA0ba	12.8	12.8	16.3	19.0
DAA0aa	23.6	20.7	20.7	0.5
DAA1	14.8	13.0	15.3	1.8
DAA2 – DAA3	26.1	23.1	24.9	4.8
DAA4 – DAA6	52.3	49.7	53.5	3.3
DAA7 – DAA12	43.5	39.5	40.5	2.1
Mortality (Daily average number of dead worker bees, drones and pupae in front of the hive per experimental group)				
Worker bees, DAA-1	1.5	1.5	0.5	2.0
Worker bees, DAA0ba	3.0	4.5	3.0	3.5
Worker bees, DAA0aa – DAA1	0.0	1.0	4.5	1440
Worker bees, DAA2 – DAA3	1.0	5.5	2.5	159.0
Worker bees, DAA4 – DAA6	2.5	3.2	4.3	58.5
Worker bees, DAA7 – DAA12	1.1	0.5	1.8	22.1
Worker bees, DAA13 – DAA21	0.4	0.4	0.6	0.2
Worker bees, DAA22 – DAA28	0.8	1.0	1.0	0.9
Drones, DAA-1 – DAA0ba	0.0	0.0	0.0	0.0
Drones, DAA0aa – DAA28	0.0	0.0	0.0	0.0
Pupae, DAA-1	0.0	0.0	0.0	0.0
Pupae, DAA0ba	0.0	0.0	0.0	0.0
Pupae, DAA0aa – DAA1	0.0	0.5	0.0	0.0
Pupae, DAA2 – DAA3	0.0	0.5	0.0	0.0
Pupae, DAA4 – DAA6	0.0	2.3	0.2	0.0
Pupae, DAA7 – DAA12	0.3	15.8	0.0	1.0
Pupae, DAA13 – DAA21	0.0	6.4	0.0	0.0
Pupae, DAA22 – DAA28	0.0	4.2	0.0	0.1

DAA = Day after application; DAA0ba = Day of application, before application; DAA0aa = Day of application, after application

Foraging activity after application was comparable in the control, test item (a.i. BYI 02960) and the toxic reference Insegar (a.i. fenoxycarb), while foraging activity decreased strongly to very low levels in Actara (a.i. thiamethoxam) group. Foraging activity in the Actara (a.i. thiamethoxam) group had not recovered during the entire confinement period. In the test item group, foraging activity was slightly reduced immediately after the treatment when compared to control, but recovered fully within 1 - 2 hours after treatment and followed the control group at all points in time thereafter.

While worker bee mortality after application was comparably low in the control, in the test item (a.i. BYI 02960) and in the Insegar (a.i. fenoxycarb) group at any point in time during the study, worker bee mortality was strongly increased in the Actara (a.i. thiamethoxam) group. Mortality of worker bees in the Actara (a.i. thiamethoxam) group remained on an elevated level compared to control until DAA11.

In none of the experimental groups there was any conspicuous drone mortality.

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In the Insegar group (a.i. fenoxycarb), where number of dead pupae was considerably increased from DAA5 until at least DAA21 (potentially until DAA28), in the other experimental groups only some single dead pupae were found.

In the Actara (a.i. thiamethoxam) group, the treatment resulted at least in transient effects on hive weight development, the extent of nectar- and pollen stores, on larval- and pupal abundance as well as on population development. In the Insegar (a.i. fenoxycarb) group, the treatment resulted at least in transient effects on larval- and pupal abundance as well as on population development.

No distinct differences were found when comparing the extent of nectar- and pollen stores, egg laying activity, larval and pupal abundance, colony strength, hive weight development and overall hive vitality in the test item treatment group with control performance at any point in time after application.

C. Validity criteria

The test was considered valid because a detectable effect of the reference item (especially Actara) was found (e. g. high adult bee mortality).

CONCLUSION

The results of the study show that BYI 02960 can be applied via foliar application at a rate corresponding to 150 g a.i./ha into a full-flowering and highly bee attractive crop, as represented here by *Phacelia tanacetifolia*, during honey bees actively foraging on the crop, without adverse effects on mortality, foraging activity, behaviour, brood-, food-, and population development as well as on overall hive vitality.

Report:	**KHA1 10.4.7/03; [redacted] (2012)**
Title:	BYI 02960 SL 200 G: A Semi-Field Study in Germany 2009 to Evaluate Effects of Spray Applications in *Phacelia tanacetifolia* on the Honeybee *Apis mellifera* L. (Hymenoptera, Apidae)
Report No:	S09-00854
Document No:	M-425576-01-2
Guidelines:	**OEPP/EPPO Guideline No. 170 (3), 2001**
Deviations:	**None**
GLP:	**Yes (certified laboratory)** **Non-GLP record: air temperature and relative air humidity (daily min/max values, respectively)**

Executive summary

The effects of BYI 02960 SL 200 G were tested on the honeybee (*Apis mellifera* L.) under confined semi-field conditions following the OEPP/EPPO guideline No. 170(3), 2001; Guideline for the efficacy evaluation of plant protection products – Side effects on honey bees, with modifications.

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The aim of the study was to evaluate potential effects of spray applications of BYI 02960 SL 200 G (Batch ID: 2009-001253; Sample description: FAR01438-00; Specification No.: 102000021884; Analysed content of a.i. BYI 02960: 17.0% w/w, 199.8 g/L) on the honeybee as well as the residues resulting from the application in bee products and in the crop.

The crop used for this semi-field study was *Phacelia tanacetifolia*, the study was conducted in Germany ([redacted]).

This study included three treatment groups with three replicates (tunnels) each: one tap-water control group (C), one test-item group (T) and one reference item group (R). In the test item treatment group, the crop was sprayed 6 days before set-up of the hives in the tunnels at BBCH 58 (end of inflorescence emergence, flower buds visible, but still closed; 1st test item application) and ten days later, at BBCH 65 (full-flowering; 2nd test item application), during honeybees actively foraging on the crop under confined conditions; the application rate of the test item corresponded to 200 g BYI 02960 a.i./ha for both applications.

The honeybees in the control and test item treatment remained 16 days in the tunnels. The exposure period of the reference item hives was only 14 days due to the very bad conditions of the bees (marginal brood cells, starved bees in cells and a lot of dead bees in the bottom of each R hive). Concurrently to the 2nd test item application, tap water was applied in the control group and Perfekthion EC 400 was applied at a rate of 400 g dimethoate a.i./ha in the reference item group. All applications were made with a spray volume of 200 L/ha.

The colony size at set-up was in the range of approximately 4000 – 7000 bees. Set up of the beehives was 4 days before the 2nd application in T and the concurrent applications in C and R, respectively.

One day before set-up of the colonies in the tunnel tents, the first colony assessment was performed. Four further colony assessments were conducted, then at weekly intervals. Overall, the colonies were assessed once before, once during and three times after the end of the confined exposure phase. Mortality assessments started 6 days before the 2nd test item application and continued on a daily basis until the re-location of the bee hives 9 (in the reference item group) and 11 days (in the control and test item group) after the 2nd test item application.

Flight intensity assessments started three days before the 2nd test item application and continued on a daily basis until the 9th (in the reference item group) and 11th (in the control and test item group) day after the 2nd test item application. Residue samples were taken in the test item treatment group and in the control group, respectively, on the day of the 2nd test item application (after application; flowers) and seven days after the 2nd test item application (flowers, pollen, nectar). The analytical phase was conducted at Bayer CropScience AG, [redacted], Germany.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

Overall conclusion:

A pre-flowering foliar application of the test item, corresponding to 200 g BYI 02960 a.i./ha to a highly bee-attractive crop (*Phacelia tanacetifolia*), was followed by a further foliar application of the test item, again corresponding to 200 g BYI 02960 a.i./ha, during full-flowering with honey bees actively foraging on the crop.

This application scenario did not cause treatment-related adverse effects on mortality, on brood and food development as well as on colony vitality under forced exposure conditions.

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A slight repellent effect of the test item was observed on the day of the 2nd test item application, after the treatment, as well as on DAA1 and DAA2.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	BYI 02960 SL 200 G
Specification number:	102000021884
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear, dark brown liquid
Batch No.:	2009-001253
Sample description:	FAR01438-00
Material number:	79718845
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.0 % w/w, 199.8 g/L according to certificate of analysis
Density:	1.175 g/mL at 20 °C
Stability of test compound:	Expiry date: 20.03.2010, when stored at 25 ± 5°C (also acceptable from +2 to +30°C)
Control:	Untreated water control (200 L/ha)
Reference Item:	
The information concerning the reference item according to the substance container label and data sheet:	
Name:	Perfekthion EC 400 (BAS 152 11 I)
Manufacturer:	[redacted]
Batch No.:	FRE-000627
Nominal content of active ingredient:	Dimethoate: 400 g/L
Analytical content of active ingredient:	Dimethoate: 422.4 g/L according to certificate of analysis
Type of formulation:	EC
Chemical state and description:	Liquid, blue
Density:	1.076 g/cm³
Solubility:	In water: emulsifiable
Stability:	Expiry date: 31.10.2009, when stored in original container, in refrigerator (4 ± 4 °C), in the dark

2. Test organisms

Species:	*Apis mellifera*
Common name:	Honey bee
Source:	Small, healthy honey bee colonies with five combs from one breeding line. Colony size: approx. 4000 – 7000 bees / colony at set-up

B. Study design and methods

1. In life dates: July 9 to August 17, 2009

2. Experimental treatments

The semi-field test was located in [redacted], Federal state of Baden-Wuerttemberg, Germany, and was conducted in tunnels located on a field of flowering *Phacelia tanacetifolia*, a surrogate crop specifically recommended in guideline OEPP/EPPO No. 170 (3) for tunnel testing.

This study included three treatment groups with three replicates (tunnels) each: one tap-water control group (C), one test-item group (T) and one reference item group (R). In the test item treatment group, the crop was sprayed 6 days before set-up of the hives in the tunnels at BBCH 58 (end of inflorescence emergence, flower buds visible, but still closed; 1st test item application) and ten days later, at BBCH 65 (full-flowering; 2nd test item application), during honeybees actively foraging on the crop under confined conditions; the application rate of the test item corresponded to 200 g BYI 02960 a.i./ha for both applications.

Table 10.4.7- 3: Treatment groups, application rates and spray volume

Treatment Group	Code	Time of application	Application rate [g a.i./ha]*	[g product/ha]	Spray Volume
Control (tap water)	C	DAA0, BBCH 65	None	None	200 L/ha
Test item (BYI 02960 SL 200 G)	T	DAA-10, BBCH 58	200	1 L	200 L/ha
		DAA0, BBCH 65	200	1 L	200 L/ha
Reference item	R	DAA0, BBCH 65	400	1 L	200 L/ha

DAA = days after 2nd test item application
* Calculation based on the nominal content of a.i.

3. Observation and measurements

The honeybees in the control and test item treatment remained 16 days in the tunnels. The exposure period of the reference item hives was only 14 days due to the very bad conditions of the bees (marginal brood cells, starved bees in cells and a lot of dead bees in the bottom of each R hive). Concurrently to the 2nd test item application, tap water was applied in the control group and Perfekthion EC 400 was applied at a rate of 400 g dimethoate a.i./ha in the reference item group. All applications were made with a spray volume of 200 L/ha.

The colony size at set-up was in the range of approximately 4000 – 7000 bees. Set up of the beehives was 4 days before the 2nd application in T and the concurrent applications in C and R, respectively.

One day before set-up of the colonies in the tunnel tents, the first colony assessment was performed. Four further colony assessments were conducted, then at weekly intervals. Overall, the colonies were assessed once before, once during and three times after the end of the confined exposure phase. Mortality assessments started 6 days before the 2nd test item application and continued on a daily basis until the re-location of the bee hives 9 (in the reference item group) and 11 days (in the control and test item group) after the 2nd test item application.

Flight intensity assessments started three days before the 2nd test item application and continued on a daily basis until the 9th (in the reference item group) and 11th (in the control and test item group) day after the 2nd test item application. Residue samples were taken in the test item treatment group and in the control group, respectively, on the day of the 2nd test item application (after application; flowers) and seven days after the 2nd test item application (flowers, pollen, nectar). The analytical phase was conducted at Bayer CropScience AG, [redacted], Germany.

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The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Total and mean number of dead bees on the linen sheets in tunnel tents and in the dead bee traps;
- Flight intensity (mean number of forager bees/m^2 and treatment group on *P. tanacetifolia* before as well as after the 2nd application in T and the concurrent applications in C and R, respectively);
- Behaviour of the bees in the crop and around the hive;
- Condition of the colonies and development of the bee brood (number of bees (strength), mean values of the different brood stages per colony and assessment date).

RESULTS AND DISCUSSION

A. Analytical Findings

For details of the analytical results as obtained by analysing samples of flowers of *Phacelia tanacetifolia,* nectar and pollen reference is made to the Analytical Phase Report, which is attached to the study report.

B. Biological Findings

<u>Honeybee mortality:</u>

The mean daily mortality per treatment group before set up (DAA-6 to -4) was 10.3, 16.0, and 4.4 dead bees for C, T, and R, respectively. After set-up of the colonies inside the tunnels until the day of the 2nd test item application (DAA-3 to 0ba), the mean mortality value was 8.3, 7.9, and 8.4 dead bees/day for the treatment group C, T, and R, respectively. On the day of the 2nd test item application (DAA0aa), after application, the mean number of dead bees was recorded to be 2.7, 8.3, and 613.7 for C, T, and R, respectively. Only on DAA1, the mean mortality in the test item treatment group was slightly, but statistically significantly higher as in the control group (t-test, method pooled, one-sided, $\alpha = 0.05$; number of dead bees: 4.0, 18.0, and 290.7 for C, T, and R, respectively). In the total post application period, the mean daily mortality was recorded to be 10.4, 14.3, and 151.2 for C, T, and R, respectively. The high mean number of dead bees in the reference item group during the post application period shows the validity of the test and was statistically significantly increased during the whole exposure period (t-test, method pooled, two-sided, $\alpha = 0.05$).

Overall, it can be concluded that BYI 02960, when applied according to the application scenario as outlined above, comprising an application corresponding to 200 g BYI 02960 a.i./ha during full-flowering of a highly bee-attractive crop (*Phacelia tanacetifolia*) with honey bees actively foraging on the crop under forced exposure conditions, does not cause treatment-related adverse effects on mortality.

Table 10.4.7- 4: Mean mortality and flight intensity values of the control group C, the test item group T and the reference group R prior to and after the 2nd application in T

Test item	BYI 02960 SL 200 G		
Test object	*Apis mellifera*		
Start of confining honeybee colonies in tunnel tents	T: 6 days after 1st test item application, 4 days before 2nd test item application (16 days in tunnel) C: 4 days before application (16 days in tunnel) R: 4 days before application (14 days in tunnel)		
Treatment group	Control **(C)**	BYI 02960 **(T)**	Reference item **(R)**
Application rate	--	1 × 200 g BYI 02960 a.i./ha at BBCH 58 and 65, respectively	1 × 400 g dimethoate a.i./ha at BBCH 65
Mean mortality DAA-6 to -4 [dead bees/day]	10.3	16.0	4.4
Mean mortality DAA-3 to 0ba [dead bees/day]	8.3	7.9	8.4
Mean mortality DAA-6 to 0ba [dead bees/day]	9.2	11.4	6.7
Mean mortality DAA0aa [dead bees/day]	2.7	8.3	613.7*
Mean mortality DAA0aa to 11 [dead bees/day]	10.4	14.3	151.2* 1)
Daily mean flight intensity DAA-3 to 0ba [bees/m²]	6.2	6.2	6.8
Daily mean flight intensity DAA0aa to 11 [bees/m²]	15.7	15.3	0.8** 1)

DAA = Days after 2nd test item application Ba = before application aa = after application
* = statistically significantly different to C (higher) ** = statistically significantly different to C (lower)
1) = due to the very bad conditions of the R hives, assessments were stopped on DAA9 by agreement with the sponsor and no further assessment took place

Honeybee flight intensity:

After set-up of the colonies inside the tunnels until the day of 2nd test item application (DAA-3 to 0ba), the mean daily flight intensity was 6.2, 6.2 and 6.8 honey bees/m² in C, T, and R, respectively.

On the day of the 2nd test item application (DAA0aa) the mean flight intensity across 7 assessments within a period of about 7 hours after application was 9.7, 6.4 and 2.9 honeybees/m² in the C, T and R respectively. In comparison to C, the mean flight intensity was slightly, but statistically significantly reduced in T and severely reduced in R. On DAA1 the flight intensity in T was lower compared to the control group and statistically significantly reduced on DAA2. Regarding the post-application period from DAA3 onwards, the flight intensity in the test item treatment was nearly on the same level as in the control.

During the entire post application period, the daily mean number of honeybees/m² was statistically significantly reduced in R compared to C (t-test, method pooled, one-sided, $\alpha = 0.05$).

The mean flight intensity in the total post application period was calculated to be 15.7 honeybees/m² in C, followed by T with 15.3 and R with only 0.8 honeybees/m².

Overall, a slight repellent effect of the test item was indicated by a reduced flight intensity on the day of the 2nd test item application (DAA0aa) as well as during the first two days thereafter (DAA1 - 2). From DAA3 onwards, the flight intensity in the test item treatment was comparable to the control.

Strength of the Colonies

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The mean number of bees assessed before set-up of the hives (first colony assessment) in the tunnels revealed a comparable colony strength in treatment groups C and T, with an average of 5221 bees/hive in C [range: 4188 – 6250] and 5063 bees/hive in T [range: 3938 – 7313]. In the reference item treatment group, the mean number of bees was 5104 bees/hive with a range of 4000 – 6250.

At the second colony assessment, during the confined exposure period, on DAA7, the mean number of bees was higher in the treatment groups C and T, whereas the mean number of bees in the reference item group was reduced (C: 6438, T: 5854, R: 3188). At the subsequent colony assessment, after the end of the confined exposure period outside the tunnels on DAA13 (third colony assessment), the mean number of bees further increased to 6896 bees/hive for C and 6750 bees/hive for T. At the two following colony assessments on DAA21 (fourth colony assessment) and DAA29 (fifth colony assessment), the mean number of bees for C and T was slightly decreased compared to the third colony assessment (C: 5771 bees/colony on DAA21 and 5229 bees/colony on DAA29; T: 5167 bees/colony on DAA21 and 6000 bees/colony on DAA29).

The development of colony strength was comparable between C and T throughout the study period and showed the fluctuations which are typical for this endpoint. Overall, no test item related adverse effects on colony strength were observed.

Development of Brood

The mean abundance of brood (sum of cells containing eggs, larvae, and pupae) assessed before set-up of the hives (first colony assessment) was 10933 cells/hive for C, 13267 cells/hive for T and 10733 cells/hive for R.

At the second colony assessment, during the confined exposure period, on DAA7, the mean abundance of brood in C and T decreased slightly, but synchronously (9000 cells/hive for C and 10133 cells/hive for T), whereas the mean abundance of brood in R decreased strongly to 3200 cells/hive. After one honey bee brood cycle, i.e. 3 weeks after the 2nd test item application (fourth colony assessment, DAA21), the mean abundance of brood in C and T was almost identical (10800 cells/hive for C and 10400 cells/hive for T), the same holds true for the last colony assessment (fifth colony assessment, DAA29: 12733 cells/hive for C and 14000 cells/hive for T).

The brood development was comparable between the control and the test item treatment colonies, the differences were within the range of natural variation. No test item related adverse effects on brood development were observed.

Development of the Food Storage Area

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The mean extent of food stores in the colonies (sum of cells containing nectar and pollen) assessed before set-up of the hives (first colony assessment) was 8600 cells/hive for C, 9867 cells/hive for T and 9067 cells/hive for R. At the second colony assessment on DAA7, the mean extent of food stores in C, T and R was found to have decreased to 5800 cells/hive for C, 6333 cells/hive for T and 4733 cells/hive for R. At the subsequent colony assessments, two days after the confined exposure period outside the tunnels on DAA13 (third colony assessment), the mean extent of food stores in the C and T colonies has further decreased to 4733 and 4133 cells containing food, respectively. On DAA21 (fourth colony assessment), the mean extent of food stores in the C and T colonies increased to 6600 and 7267 cells containing food, respectively. On the last colony assessment on DAA29, the mean extent of food stores in the C and T colonies decreased to 5467 and 4867 cells containing food, respectively.

The observed decrease in food stores in both, test item treatment and control during confinement as well as the subsequent increase can be considered as typical for this type of study. The decrease of the mean extent of food stores on the last assessment day (DAA29) can be explained by the advanced season. No test-item related adverse effects on the development of the food storage area were observed.

Behaviour of the Bees

Clustering at the hive entrance on DAA1 in hive C3 and on DAA10 in hive T1 was observed. No further abnormal behaviour was recorded in the control or in the test item treatment group,. After the application of the reference item, a strong repellence effect (no or low flight activity on the crop) as well as several bees with obvious signs of intoxication were observed in the reference item group.

Residue Analysis

Details of the analytical results as obtained by analysing samples of flowers of *Phacelia tanacetifolia,* nectar and pollen, are given in an Analytical Phase Report, which is attached to the report.

C. Validity criteria

The test was considered valid because a detectable effect of the reference item was found (e. g. high adult bee mortality).

CONCLUSION

A pre-flowering foliar application of the test item, corresponding to 200 g BYI 02960 a.i./ha to a highly bee-attractive crop (*Phacelia tanacetifolia*), was followed by a further foliar application of the test item, again corresponding to 200 g BYI 02960 a.i./ha, during full-flowering with honey bees actively foraging on the crop.

This application scenario did not cause treatment-related adverse effects on mortality, on brood and food development as well as on colony vitality under forced exposure conditions.

A slight repellent effect of the test item was observed on the day of the 2nd test item application, after the treatment, as well as on DAA1 and DAA2.

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Report:	**KIIIA1 10.4.7/04; [redacted] (2012)**
Title:	BYI 02960 SL 200 G: A Semi-Field Study in Denmark 2010 to Evaluate Effects of Spray Applications in *Phacelia tanacetifolia* on the Honeybee *Apis mellifera* L. (Hymenoptera, Apidae)
Report No:	S10-01954
Document No:	M-423156-01-2
Guidelines:	**OEPP/EPPO Guideline No. 170 (3), 2001**
Deviations:	**None**
GLP:	**Yes (certified laboratory)**

Executive summary

The effects of BYI 02960 SL 200 G were tested on the honeybee (*Apis mellifera* L.) under confined semi-field conditions following the OEPP/EPPO guideline No. 170(3), 2001.

The aim of the study was to evaluate potential effects of spray applications of BYI 02960 SL 200 G (Batch No: 2010-001067; TOX08907-00; Specification No: 102000021884-01; Analysed content of a.i. BYI 02960: 17.1% w/w, 201.0 g/L) on the honeybee as well as the residues resulting from the application in bee products and in the crop.

The crop used for this semi-field study was *Phacelia tanacetifolia*, the study was conducted in Denmark.

This study included 3 treatment groups with 3 replicates (tunnels) each: one tap-water control group (C), one test-item group (T) and one reference item group (R).

In T, the 1st test item application (followed by immediate soil incorporation) was carried out on bare soil (BBCH 00) at the day of sowing, before the actual drilling of *Phacelia tanacetifolia*-seeds at a rate of 300 g a.i./ha. Thereafter, in the test item treatment group, the crop was sprayed 4 days before set-up of the hives in the tunnels at BBCH 60 (beginning of flowering, first flowers open; 2nd test item application, 200 g a.i./ha) and seven days later, at BBCH 65 (full-flowering; 3rd test item application, 200 g a.i./ha), during honeybees actively foraging on the crop under confined conditions.

The honeybees remained 10 days in the tunnels. Concurrently to the 3rd test item application, tap water was applied in the control group and Perfekthion EC 400 (400 g dimethoate/ha) in the reference item group.

The colonies were assessed once before, once at the end and three times after the confined exposure phase. Mortality and flight intensity assessments started before the 3rd test item application and continued on a daily basis until the re-location of the bee hives 7 days after the 3rd test item application. Residue samples were taken in the test item treatment group and in the control group, respectively, on the day of the 3rd test item application (after application; flowers) and seven days after the 3rd test item application (flowers, pollen, nectar).

<u>Overall conclusion:</u>

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A soil application of the test item, corresponding to 300 g BYI 02960 a.i./ha, followed by immediate soil incorporation and the subsequent sowing of *Phacelia*-seeds was performed in combination with a pre-flowering foliar application of the test item, corresponding to 200 g BYI 02960 a.i./ha, followed by a further foliar application of the test item, corresponding to 200 g BYI 02960 a.i./ha during full-flowering of a highly bee-attractive crop (*Phacelia tanacetifolia*) with honey bees actively foraging on the crop. This application scenario did not cause treatment-related adverse effects on mortality and no effects on brood and food development or on colony vitality under forced exposure conditions.

A slight repellent effect of the test item was observed on the day of the 3rd test item application, after the treatment, as well as on DAA1 and from DAA3 to 5.

MATERIAL AND METHODS

A. Material

1. Test material

<u>Test item:</u>	BYI 02960 SL 200 G
Specification number:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Batch No.:	2010-001067
Sample description:	TOX08907-00
Material number:	79718845
Nominal content of active substance:	BYI 02960: 200 g/L
Analytical content of active substance:	BYI 02960: 17.1% w/w, 201.0 g/L acc. to certificate of analysis
Density:	1.175 g/mL at 20 °C
Stability of test compound:	Expiry date: 14.06.2012, when stored at 25 ± 5°C in original container in the dark (also acceptable from +2 to +30°C)
<u>Control:</u>	Untreated water control (200 L/ha)

<u>Reference Item:</u>

The information concerning the reference item according to the substance container label and data sheet:

Name:	Perfekthion EC 400 (BAS 152 11 I)
Manufacturer:	[redacted]
Batch No.:	90924-06
Nominal content of active ingredient:	Dimethoate: 400 g/L
Analytical content of active ingredient:	Dimethoate: 414.8 g/L according to certificate of analysis
Certificate of Analysis Study Code:	
Type of formulation:	EC
Chemical state and description:	Liquid, blue
Density:	1.074 g/cm^3
Solubility:	In water: emulsifiable
Stability:	Expiry date: 07.10.2011, when stored in original container, in refrigerator (4 ± 4 °C), in the dark

2. Test organisms

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Species:	*Apis mellifera*
Common name:	Honey bee
Source:	Small, healthy honey bee colonies with ten combs from one breeding line. Colony size: approx. 1000 – 9000 bees / colony at set-up, 3 days before the 3rd application in T (DAA-3)

B. Study design and methods

1. In life dates: May 27, 2010 to June 16, 2011

2. Experimental treatments

The semi-field test was located in [redacted], near [redacted], Region Syddanmark, Denmark, and was conducted in tunnels located on a field of flowering *Phacelia tanacetifolia*, a surrogate crop specifically recommended in guideline OEPP/EPPO No. 170 (3) for tunnel testing.

This study included three treatment groups with three replicates (tunnels) each: one tap-water control group (C), one test-item group (T) and one reference item group (R). In T, the 1st test item application (followed by immediate soil incorporation) was carried out on bare soil at the day of sowing, before the actual drilling of *Phacelia tanacetifolia*-seeds. The rate of the 1st test item application (BBCH 00) corresponded to 300 g a.i./ha. Thereafter, in the test item treatment group, the crop was sprayed 4 days before set-up of the hives in the tunnels at BBCH 60 (beginning of flowering, first flowers open; 2nd test item application) and seven days later, at BBCH 65 (full-flowering; 3rd test item application), during honeybees actively foraging on the crop under confined conditions; the application rate of the test item corresponded at BBCH 60 and 65 to 200 g BYI 02960 a.i./ha, respectively.

Table10.4.7- 5: Treatment groups, application rates and spray volume

Treatment group	Code	Time of application	Application rate [g a.i./ha]*	Application rate [g product/ha]	Spray volume
Control (tap water)	C	DAA0 BBCH 65	**None**	**None**	**200 L/ha**
Test item (BYI 02960 SL 200 G)	T	On the day of sowing, before sowing, BBCH 00	**300**	**1.5 L**	**200 L/ha**
		DAA -7, BBCH 60	200	**1 L**	**200 L/ha**
		DAA0, BBCH 65	200	**1 L**	**200 L/ha**
Reference item	R	DAA0, BBCH 65	**400**	**1 L**	**200 L/ha**

DAA = days after 3rd test item application in T
* Calculation based on the nominal content of a.i.

3. Observation and measurements

Set-up of the beehives was 3 days before the 3rd application in T and the concurrent applications in C and R, respectively (set-up of hives in the evening after bee flight). The honeybees remained for 10 days in the tunnels. Concurrently to the 3rd test item application, tap water was applied in the control group and Perfekthion EC 400 was applied at a rate of 400 g dimethoate a.i./ha in the reference item group. All applications were made with a spray volume of 200 L/ha. The colony size at set-up was in the range of approximately 1000 – 9000 bees.

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At the day of set-up of the colonies in the tunnel tents, the first colony assessment was performed. Four further colony assessments were conducted, at weekly intervals. Overall, the colonies were assessed once before, once at the end and three times after the end of the confined exposure phase. Mortality assessments started 6 days before the 3rd test item application and continued on a daily basis until the re-location of the bee hives 7 days after the 3rd test item application. Flight intensity assessments started two days before the 3rd test item application and continued on a daily basis until the 7th day after the 3rd test item application. Residue samples were taken in the test item treatment group and in the control group, on the day of the 3rd test item application (after application; flowers) and seven days after the 3rd test item application (flowers, pollen, nectar).

The analytical phase was conducted at Bayer CropScience AG, [redacted], Germany.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Total and mean number of dead bees on the linen sheets in tunnel tents and in the dead bee traps;
- Flight intensity (mean number of bees/m^2 and treatment group on *P. tanacetifolia* before as well as after application);
- Behaviour of the bees in the crop and around the hive;
- Condition of the colonies and development of the bee brood (number of bees (strength), mean values of the different brood stages per colony and assessment date).

RESULTS AND DISCUSSION

A. Analytical Findings

For details of the analytical results as obtained by analysing samples of flowers of *Phacelia tanacetifolia,* nectar and pollen as well as soil samples reference is made to the Analytical Phase Report, which is attached to the study report.

B. Biological Findings

Honeybee Mortality:

The mean daily mortality per treatment group before set up of the colonies inside the tunnels (DAA-6 to -3) was 1.2, 2.4, and 2.2 dead bees for C, T, and R, respectively. After set-up until the day of the 3rd test item application (DAA-2 to 0ba), the mean mortality value was 4.2, 8.0, and 12.9 dead bees/day for the treatment group C, T, and R, respectively. On the day of the 3rd test item application, after application (DAA0aa), the mean number of dead bees was recorded to be 3.0, 12.0, and 335.7 for C, T, and R, respectively. Mean mortality in treatment group R on DAA0aa was statistically significantly higher than in the control group. In the total post application period (DAA0aa to 7), the mean daily mortality was recorded to be 3.0, 14.9, and 142.0 for C, T, and R, respectively. Mortality values for T and R were statistically significantly higher than in C. When comparing the mean mortality before application (DAA -2 to DAA0ba) to the day of application, $Q_{M(0aa)}$ values were calculated to be 0.7, 1.5 and 26.0 for the treatment groups C, T and R, respectively. The $Q_{M(mean)}$ values were calculated to be 0.7 for C, 1.9 for T and 11.0 for R, respectively.

The high mean number of dead bees in R during the post application period shows the sensitivity of the test system and was statistically significantly increased from DAA0aa to DAA5.

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Mortality in the control group was generally slightly lower than in the test item treatment group. This holds true for the period before and after the 3rd test item application, and is in line with the, on average, more than 3-times higher number of bees in the test item treatment group as compared to the control group (determined during the 1st colony assessment on DAA-3). The higher number of bees in the test item treatment group entails a higher turnover of the stronger colonies in terms of mortality rates (at set-up, control colonies comprised on average 1337 bees [range: 816 - 2254], treatment colonies comprised on average 4274 bees [range: 1503 - 9192]). Nonetheless, except for three single days (DAA-6, DAA-2 and DAA6), the slightly higher mean mortalities in T were not statistically significant when compared to C. In general mortality values in the control group were on a low level throughout the test and the higher mean values in the treatment group were biased particularly by colony 1T with the more than 9000 bees at the time of set-up.

Overall, it can be concluded that BYI 02960, when applied according to the application scenario as outlined above, comprising an application corresponding to 200 g BYI 02960 a.i./ha during full-flowering of a highly bee-attractive crop (*Phacelia tanacetifolia*) with honey bees actively foraging on the crop under forced exposure conditions, does not cause treatment-related adverse effects on mortality.

Table 10.4.7- 6: Mean mortality and flight intensity values of the control group C, the test item group T and the reference group R prior to and after the 3rd application in T

Test item	BYI 02960 SL 200 G		
Test object	*Apis mellifera*		
Start of confining honeybee colonies in tunnel tents	T: 4 days after 2nd test item application, 3 days before 3rd test item application (10 days in tunnel) C: 3 days before application (10 days in tunnel) R: 3 days before application (10 days in tunnel)		
Treatment group	Control (C)	BYI 02960 SL 200 G (T)	Reference item (R)
Application rate	-	1 × 300 g BYI 02960 a.i./ha at BBCH 00; 1 × 200 g BYI 02960 a.i./ha at BBCH 60 and 65, respectively	1 × 400 g dimethoate a.i./ha at BBCH 65
Mean mortality DAA-6 to -3 [dead bees/day]	1.2	2.4	2.2
Mean mortality DAA-2 to 0ba [dead bees/day]	4.2	8.0	12.9
Mean mortality DAA-6 to 0ba [dead bees/day]	2.5	4.9	6.8
Mean mortality DAA0aa [dead bees/day]	3.0	12.0	335.7 *
Mean mortality DAA0aa to 7 [dead bees/day]	3.0	14.9	142.0 *
Daily mean flight intensity DAA-2 to 0ba [bees/m²]	4.1	7.3	9.6
Daily mean flight intensity DAA0aa to 7 [bees/m²]	14.5	12.7	0.9 **

DAA = Days after 3rd test item application ba = before test item application
aa = after test item application
* statistically significantly different to C (higher) ** statistically significantly different to C (lower)

Honeybee Flight Intensity

After set-up of the colonies inside the tunnels until the day of 3rd test item application (DAA-2 to 0ba), the mean daily flight activity was 4.1, 7.3, and 9.6 honeybees/m² in C, T, and R, respectively.

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On the day of the 3rd application after the application (DAA0aa), on the day after this application (DAA1) and from DAA3 to 5 a slight reduction in flight intensity was found in the test item treatment group T, when compared to the control group C (DAA0: 8.0 honeybees/m² in C compared to 6.4 honeybees/m² in T, DAA1: 6.0 honeybees/m² in C compared to 3.7 honeybees/m² in T; DAA3 to 5: 14.3 to 23.3 honeybees/m² in C compared to 11.0 to 12.3 honeybees/m² in T). Only on DAA5, the flight intensity was statistically significantly lower in T when compared to C. During the last two days of the confined exposure period (DAA6 and 7) the flight intensity in T was higher than the control with 24.3 bees/m² (DAA6) and 21.7 bees/m² (DAA7) in T as compared to 19.3 bees/m² (DAA6) and 16.3 bees/m² (DAA7) in C.

The flight activity in the reference item group was statistically significantly reduced when compared to C from DAA0aa up to DAA7 (values from 0.0 to 2.3 honeybees/m² in R). Over the entire post application period, the mean number of bees/m²/min was 14.5 for C, 12.7 for T, and 0.9 for R.

Overall, a slight repellent effect of the test item was indicated by a reduced flight intensity on the day of the 3rd test item application DAA0aa as well as on DAA1 and from DAA3 to 5.

Strength of the Colonies

The mean number of bees assessed before set-up of the hives in the tunnels (first colony assessment) revealed a more than 3-times higher colony strength in T as compared to C, with on average 1337 bees/hive in C [range: 816 - 2254], 4274 bees/hive in T [range: 1503 - 9192] and 3065 bees/hive in R [range: 1252 - 6129]. At the second brood assessment, during the confined exposure period, on DAA7, the mean number of bees equalised between the treatment groups (C: 3857, T: 3795, R: 2879). At the subsequent brood assessment, after the relocation of the hives to the monitoring site on DAA14 (third colony assessment), the mean number of bees in C and T increased to 4793 bees/hive in C and 6190 bees/hive in T. At the two following colony assessments on DAA20 (fourth colony assessment) and DAA28 (fifth colony assessment), the mean number of bees for C and T were assessed to be: 5377 bees/colony on DAA20 and 7167 bees/colony on DAA28 in C; 5920 bees/colony on DAA20 and 5733 bees/colony on DAA28 in T.

When analysing the individual colony performance in C and T during the confined exposure period in detail, it becomes obvious that all colonies in C and T, except the one single colony in T (1T) with the more than 9000 bees at the first colony assessment, increased in their number of bees during the confined exposure period. This observation can be explained by the limited amount of forage under the confined tunnel situation: whereas the available, limited forage was sufficient for the smaller colonies to even grow during confinement, the available forage was obviously not sufficient for the much stronger colonies, resulting in a decrease of colony strength. Thus, the observed decrease in the average number of bees in T during the confined exposure conditions is biased by the reduction of bees in the strongest T-colony 1T and is not related to the treatment. This conclusion is further supported when analysing the individual performance data of the colonies in C and T in terms of relative increase in colony strength during the confined exposure conditions: generally, the weaker the colony at the time of set-up in the tunnel tents, the stronger its relative increase in colony strength. Only the overall strongest colonies, (i.e. colony 1T with more than 9000 bees at the first colony assessment and colony 2R with more than 6000 bees at the first colony assessment), which were initially much stronger than all the other colonies, decreased in their colony strength during confinement.

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After the end of the confined exposure situation, the development of colony strength was comparable between the treatment groups and showed the fluctuations which are typical for this endpoint. Overall, no test item related adverse effects on colony strength were observed.

Development of Brood

The mean abundance of brood (sum of cells containing eggs, larvae, and pupae) assessed before set-up of the hives (first colony assessment) was 14067 cells/hive for C, 16733 cells/hive for T and 12667 cells/hive for R. At the second colony assessment, during the confined exposure period, on DAA7, the mean abundance of brood in C and T increased (14733 cells/hive for C and 18733 cells/hive for T), whereas the mean abundance of brood in R decreased to 8600 cells/hive. After one honey bee brood cycle, i.e. 3 weeks after the 3rd test item application (fourth colony assessment, DAA20), the mean abundance of brood in C and T had further increased (18067 cells/hive for C and 22333 cells/hive for T), the same holds true for the last colony assessment (fifth colony assessment, DAA28: 22667 cells/hive for C and 26933 cells/hive for T).

The brood development was comparable between the control and the test item treatment colonies, the differences were within the range of natural variation. No test-item related adverse effects on brood development were observed.

Development of the Food Storage Area

The mean extent of food stores in the colonies (sum of cells containing nectar and pollen) assessed before set-up of the hives (first colony assessment) was 19933 cells/hive for C, 18867 cells/hive for T and 15000 cells/hive for R. At the second colony assessment, during the confined exposure period, on DAA7, the mean extent of food stores in the colonies C, T and R decreased synchronously (14667 cells/hive for C, 14440 cells/hive for T and 13600 cells/hive for R). At the subsequent colony assessment, after the end of the confined exposure period outside the tunnels on DAA14 (third colony assessment), the mean extent of food stores in the colonies C and T increased again to 15600 cells/hive for C and 16667 cells/hive for T. At the two following colony assessments (fourth and fifth colony assessment, DAA20 and DAA28), the mean extent of food stores in the colonies C and T further increased and remained on a comparable level (DAA20: 21866 cells/hive for C and 23133 cells/hive for T; DAA28: 25733 cells/hive for C and 25067 cells/hive for T).

The observed decrease in food stores in both, treatment and control, during confinement as well as the subsequent increase can be considered as typical for this type of study. No test-item related adverse effects on the development of the food storage area were observed.

Behaviour of the Bees

No abnormal behaviour was recorded in the control group. Bees of one of the hives in the test item treatment group T were clustering at the hive entrance on the day of the 3rd test item application during the two last assessments (i.e. colony 1T with a colony strength of more than 9000 bees assessed at the first colony assessment). From DAA1 until the end of the confinement period on DAA7, no abnormal behaviour of the bees was observed in all three replicates of T. After the application of the reference item, a strong repellence effect (no or low flight activity on the crop) as well as several bees with obvious signs of intoxication were observed in the reference item group.

Residue Analysis

Details of the analytical results as obtained by analysing samples of flowers of *Phacelia tanacetifolia,* nectar and pollen as well as soil samples are given in an Analytical Phase Report, which is attached to the report.

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C. Validity criteria

The test was considered valid because a detectable effect of the reference item was found (e. g. high adult bee mortality).

CONCLUSION

A soil application of the test item, corresponding to 300 g BYI 02960 a.i./ha, followed by immediate soil incorporation and the subsequent sowing of *Phacelia*-seeds was performed in combination with a pre-flowering foliar application of the test item, corresponding to 200 g BYI 02960 a.i./ha, followed by a further foliar application of the test item, corresponding to 200 g BYI 02960 a.i./ha during full-flowering of a highly bee-attractive crop (*Phacelia tanacetifolia*) with honey bees actively foraging on the crop.

This application scenario did not cause treatment-related adverse effects on mortality and no effects on brood and food development as well as on colony vitality under forced exposure conditions.

A slight repellent effect of the test item was observed on the day of the 3rd test item application, after the treatment, as well as on DAA1 and from DAA3 to 5.

Report:	**KIIIA1 10.4.7/05; [redacted] (2012)**
Title:	BYI 02960 SL 200 G: A Semi-Field Study in Italy 2011 to Evaluate Effects of Spray Applications in *Phacelia tanacetifolia* on the Honeybee *Apis mellifera* L. (Hymenoptera, Apidae)
Report No:	S10-01955
Document No:	M-423172-01-2
Guidelines:	OEPP/EPPO Guideline No. 170 (4), 2010
Deviations:	None
GLP:	Yes (certified laboratory) Climatic data (air temperature and humidity, rainfall) were recorded by a nearby weather station (non-GLP record)

Executive summary

The effects of BYI 02960 SL 200 G were tested on the honeybee (*Apis mellifera* L.) under confined semi-field conditions following the OEPP/EPPO guideline No. 170(4), 2010.

The aim of the study was to evaluate potential effects of spray applications of BYI 02960 SL 200 G (Batch No: 2010-007173; Sample description: FAR01535-00; Specification No: 102000021884-01; Analysed content of a.i. BYI 02960: 16.9% w/w, 198.6 g/L) on the honeybee as well as the residues resulting from the application in bee products and in the crop.

The crop used for this semi-field study was *Phacelia tanacetifolia*, the study was conducted in Northern Italy.

This study included 3 treatment groups with 3 replicates (tunnels) each: one tap-water control group (C), one test-item group (T) and one reference item group (R).

In T, the 1st test item application (followed by immediate soil incorporation) was carried out on bare soil (BBCH 00) at the day of sowing, before the actual drilling of *Phacelia tanacetifolia*-seeds at a rate of 300 g a.i./ha. Thereafter, in the test item treatment group, the crop was sprayed 14 days before set-up of the hives in the tunnels at BBCH 58-61 (beginning of flowering, first flowers open; 2nd test item application, 200 g a.i./ha) and 4 days after set-up, at BBCH 63-68 (full-flowering; 3rd test item application, 200 g a.i./ha), during honeybees actively foraging on the crop under confined conditions.

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The honeybees remained 12 days in the tunnels. Concurrently to the 3rd test item application, tap water was applied in the control group and Perfekthion EC 400 (400 g dimethoate/ha) in the reference item group. All applications were made with a spray volume of 200 L/ha. The colony size at set-up was in the range of approximately 7000-13800 bees.

The colonies were assessed once before, once at the end and three times after the confined exposure phase. Mortality assessments started 9 days before the 3rd test item application and continued on a daily basis until the re-location of the bee hives to the monitoring site, 7 days after the 3rd test item application. Flight intensity assessments started four days before the 3rd test item application and continued on a daily basis until the 7th day after the 3rd test item application.

Soil samples for chemical analysis of BYI 02960 were taken directly after the first application and immediate incorporation of the test item on each tunnel plot in the test item treatment group, respectively. Further residue samples were taken in the test item treatment group and in the control group, respectively, on the day of the 3rd test item application (after application; flowers) and seven days after the 3rd test item application (flowers, nectar and wax).

Overall conclusion:

A soil application of the test item, corresponding to 300 g BYI 02960 a.i./ha, followed by immediate soil incorporation and the subsequent sowing of *Phacelia*-seeds was performed in combination with a pre-flowering foliar application of the test item, corresponding to 200 g BYI 02960 a.i./ha, followed by a further foliar application of the test item, corresponding to 200 g BYI 02960 a.i./ha during full-flowering of a highly bee-attractive crop (*Phacelia tanacetifolia*) with honeybees actively foraging on the crop.

This application scenario did not cause treatment-related adverse effects on mortality, brood and food development as well as on colony vitality throughout the entire observation time. A slight repellent effect of the test item was observed on the day of the 3rd test item application, after the treatment.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	BYI 02960 SL 200 G
Specification number:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear, orange-brown liquid
Batch No.:	2010-007173
Sample description:	FAR01535-00
Material number:	79718845
Nominal content of active substance:	BYI 02960: 200 g/L
Analytical content of active substance:	BYI 02960: 16.9% w/w; 198.6 g/L acc. to certificate of analysis
Density:	1.175 g/mL at 20°C
Stability of test compound:	Expiry date: 30.09.2012, when stored at 25 ± 5°C in original container in the dark (also acceptable from +2 to +30°C)
Control:	Untreated water control (200 L/ha)

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Reference Item:

The information concerning the reference item according to the substance container label and data sheet:

Name:	Perfekthion EC 400 (BAS 152 11 I)
Manufacturer:	[redacted]
Batch No.:	90924-06
Nominal content of active substance:	Dimethoate: 400 g/L
Analytical content of active substance:	Dimethoate: 414.8 g/L according to certificate of analysis
Type of formulation:	EC
Chemical state and description:	Liquid, blue
Density:	1.074 g/cm^3
Solubility:	In water: emulsifiable
Stability:	Expiry date: 07.10.2011, when stored in original container in refrigerator (4 ± 4 °C), in the dark

2. Test organisms

Species:	*Apis mellifera*
Common name:	Honey bee
Source:	Small, healthy honey bee colonies with 6 combs from one breeding line. Colony size: approx. 7000 – 13800 bees / colony at set-up, 4 days before 3rd application, DAA-4)

B. Study design and methods

1. In life dates: April 5 to December 14, 2011

2. Experimental treatments

The semi-field test was located in [redacted], province of Ferrara, in the region Emilia Romagna, Italy, and was conducted in tunnels located on a field of flowering *Phacelia tanacetifolia*, a surrogate crop specifically recommended in guideline OEPP/EPPO Guideline No. 170 (4) for tunnel testing.

This study included three treatment groups with three replicates (tunnels) each: one tap-water control group (C), one test item group (T) and one reference item group (R). In T, the 1st test item application (followed by immediate soil incorporation) was carried out on bare soil at the day of sowing, before the actual drilling of *Phacelia tanacetifolia*-seeds. The rate of the 1st test item application (BBCH 00) corresponded to 300 g a.i./ha. Thereafter, in the test item treatment group, the crop was sprayed 14 days before set-up of the hives in the tunnels at BBCH 58-61 (beginning of flowering, first flowers open; 2nd test item application) and four days after set-up, at BBCH 63-68 (full-flowering; 3rd test item application), during honeybees actively foraging on the crop under confined conditions. For the 2nd and 3rd application, the rate of the test item corresponded to 200 g BYI 02960 a.i./ha, respectively.

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Table 10.4.7- 7: Treatment groups, application rates and spray volume

Treatment group	Code	Time of application	Application rate [g a.i./ha]*	Application rate [g product/ha]	Spray volume
Control (tap water)	C	DAA0, BBCH 63-68	None	None	200 L/ha
Test item (BYI 02960 SL 200 G)	T	On the day of sowing, before sowing, BBCH 00	300	1.5 L	200 L/ha
		DAA-18, BBCH 58-61	200	1 L	200 L/ha
		DAA0, BBCH 63-68	200	1 L	200 L/ha
Reference item	R	DAA0, BBCH 63-68	400	1 L	200 L/ha

DAA = days after 3rd test item application in T
* Calculation based on the nominal content of a.i.

3. Observation and measurements

Set up of the beehives was 4 days before the 3rd application in T and the concurrent applications in C and R. The honeybees remained for 12 days in the tunnels. Concurrently to the 3rd test item application, tap water was applied in the control group and Perfekthion EC 400 was applied at a rate of 400 g dimethoate a.i./ha in the reference item group. All applications were made with a spray volume of 200 L/ha. The colony size at set-up was in the range of approximately 7000-13800 bees.

On the day before set-up of the colonies in the tunnel tents, the first colony assessment was performed. Four further colony assessments were conducted at weekly intervals. Overall, the colonies were assessed once before, once at the end and three times after the confined exposure phase. Mortality assessments started 9 days before the 3rd test item application and continued on a daily basis until the re-location of the bee hives to the monitoring site, 7 days after the 3rd test item application. Flight intensity assessments started four days before the 3rd test item application and continued on a daily basis until the 7th day after the 3rd test item application.

Soil samples for chemical analysis of BYI 02960 were taken directly after the first application and immediate incorporation of the test item on each tunnel plot in the test item treatment group, respectively. Further residue samples were taken in the test item treatment group and in the control group, respectively, on the day of the 3rd test item application (after application; flowers) and seven days after the 3rd test item application (flowers, nectar and wax). The analytical phase was conducted at Bayer CropScience AG, [redacted], Germany.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Total and mean number of dead bees on the linen sheets in tunnel tents and in the dead bee traps;
- Flight intensity (mean number of bees/m^2 and treatment group on *P. tanacetifolia* before as well as after application);
- Behaviour of the bees in the crop and around the hive;
- Condition of the colonies and development of the bee brood (number of bees (strength), mean values of the different brood stages per colony and assessment date).

RESULTS AND DISCUSSION

A. Analytical Findings

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For details of the analytical results as obtained by analysing samples of flowers of *Phacelia tanacetifolia,* nectar and wax as well as soil samples reference is made to the Analytical Phase Report, which is attached to the study report.

B. Biological Findings

Honeybee Mortality

The mean daily mortality per treatment group before set up (DAA-9 to -5) was 17.3, 13.6 and 14.7 dead bees for C, T, and R, respectively. After set-up of the colonies inside the tunnels until the day of the 3rd test item application (DAA-4 to 0ba), the mean mortality value was 77.6, 37.6 and 98.5 dead bees/day for the treatment group C, T, and R, respectively. On the day of the 3rd test item application, after application (DAA0aa), the mean number of dead bees was recorded to be 26.0, 31.3 and 1125.7 dead bees/day for C, T, and R, respectively. In the total post application period, the mean daily mortality was recorded to be 74.4, 49.2 and 318.3 dead bees/day for C, T, and R, respectively. When comparing the mean mortality before application (DAA-4 to DAA0ba) to the day of application, $Q_{M(0aa)}$ values were calculated to be 0.3, 0.8 and 11.4 for the treatment groups C, T and R, respectively. The $Q_{M(mean)}$ values were calculated to be 1.0 for C, 1.3 for T and 3.2 for R, respectively. The high mean number of dead bees in R during the post application period shows the sensitivity of the test system and was statistically significantly increased from DAA0aa to DAA3.

The observed mortality per individual colony entails generally a higher turnover of the stronger colonies as compared to the weaker colonies.

No statistically significant differences between the daily mortality values in the control and the test item treatment group were detected throughout the entire assessment period.

Overall, it can be concluded that BYI 02960, when applied according to the application scenario as outlined above, comprising an application corresponding to 200 g BYI 02960 a.i./ha during full-flowering of a highly bee-attractive crop (*Phacelia tanacetifolia*) with honey bees actively foraging on the crop under forced exposure conditions, does not cause treatment-related adverse effects on mortality.

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Table 10.4.7- 8: Mean mortality and flight intensity values of the control group C, the test item group T and the reference group R prior to and after the 3rd application in T

Test item	BYI 02960 SL 200 G		
Test object	*Apis mellifera*		
Start of confining honeybee colonies in tunnel tents	T: 14 days after 2nd test item application, 4 days before 3rd test item application (12 days in tunnel) C: 4 days before application (12 days in tunnel) R: 4 days before application (12 days in tunnel)		
Treatment group	Control (C)	BYI 02960 SL 200 G (T)	Reference item (R)
Application rate	-	1 × 300 g BYI 02960 a.i./ha at BBCH 00; 1 × 200 g BYI 02960 a.i./ha at BBCH 58-61 and 63-68, respectively	1 × 400 g dimethoate a.i./ha at BBCH 63-68
Mean mortality DAA-9 to -5 [dead bees/day]	17.3	13.6	14.7
Mean mortality DAA-4 to 0ba [dead bees/day]	77.6	37.6	98.5
Mean mortality DAA-9 to 0ba [dead bees/day]	47.4	25.6	56.6
Mean mortality DAA0aa [dead bees/day]	26.0	31.3	1125.7*
Mean mortality DAA0aa to 7 [dead bees/day]	74.4	49.2	318.3*
Daily mean flight intensity DAA-4 to 0ba [bees/m²]	7.4	8.0	8.5
Daily mean flight intensity DAA0aa to 7 [bees/m²]	10.3	7.8	3.5**

DAA = Days after 3rd test item application ba= before 3rd test item application
aa= after 3rd test item application
* statistically significantly different to C (higher) ** statistically significantly different to C (lower)

Honeybee Flight Intensity

After set-up of the colonies inside the tunnels until the day of the 3rd test item application (DAA-4 to 0ba), the mean daily flight activity was 7.4, 8.0, and 8.5 honeybees/m² in C, T and R, respectively.

On the day of the 3rd test item treatment, the mean flight intensity across 7 assessments within a period of 6 hours after application was 13.3 honeybees/m² in the control group. In comparison, flight intensity was slightly, but statistically significantly reduced in T with 8.7 honeybees/m² and severely reduced in R with 3.3 honeybees/m². The slight reduction of flight intensity in T was caused by a repellent effect from approximately 60 minutes up to three hours after application. About 6 hours after application, the mean flight intensity in T was with 13.3 honeybees/m² on the same level as in C with 15.0 honeybees/m².

Regarding the post-application period from DAA0aa to DAA7, the mean flight intensity in C was calculated to be 10.3 honeybees/m², followed by T with 7.8 and R with only 3.5 honeybees/m². The lower mean numbers in T were caused by colony 3T, where a lower flight activity was recorded throughout the post-application period (mean: 5.2 honeybees/m²). The mean flight activity of the two other T-colonies (1T and 2T) was recorded during the same time period to be 9.5 and 8.7 honeybees/m², which can be regarded to be on the same level as the mean value in C (10.3 honeybees/m²).

Strength of the Colonies

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The mean number of bees assessed before set-up of the hives (first colony assessment, DAA-5) in the tunnels revealed a comparable colony strength in the treatment groups C and T, with an average of 8280 bees/hive in C [range: 7130 - 9200] and 8740 bees/hive in T [range: 7130 - 9660]. In the reference item treatment group R, the mean number of bees was higher with 10350 bees/hive [range: 7590 - 13800]. This was caused by the strong colony 2R [13800 bees/hive].

At the second colony assessment, at the end of the confined exposure period on DAA7, the mean number of bees recorded was still 8280 bees/colony in C, 7590 bees/colony in T and only 6785 bees/colony in R. At the third colony assessment on DAA14, the mean number of bees in C, T and R increased to 9430, 10887 and 10043 bees/colony. At the two following colony assessments on DAA21 (fourth colony assessment) and DAA28 (fifth colony assessment), the mean number of bees in C, T and R were assessed to be: 11040 bees/colony on DAA21 and 10197 bees/colony on DAA28 in C; 9583 bees/colony and 9737 bees/colony in T and 9890 bees/colony and 9660 bees/colony in R.

When analysing the individual colony performance in C and T during the confined exposure period in detail, it becomes obvious that all colonies in C and T with initially about 9000 bees at the first colony assessment, decreased in their number of bees during the confined exposure period. This observation can be explained by the limited food supply under the confined tunnel situation. Whereas the available, limited food supply was sufficient for the smaller colonies to even grow after application during confinement (2C: 7130→8970; 1T: 7130→ 8280), the available food supply was obviously not sufficient for the stronger colonies, resulting in a decrease of colony strength (1C: 9200→8280; 3C: 8510→7590 and 2T: 9660→7590; 3T: 9430→6900).

Thus, it can be concluded that the development of colony strength up to the last colony assessment (DAA28) was comparable between C and T with fluctuations which are typical for this endpoint. Overall, no test-item related adverse effects on colony strength were observed.

Development of Brood

The mean abundance of brood (sum of cells containing eggs, larvae, and pupae) assessed before set-up of the hives (first colony assessment) was 31020 cells/hive for C, 28981 cells/hive for T and 26547 cells/hive for R. At the second colony assessment, at the end of the confined exposure period on DAA7, the mean abundance of brood in C, T and R was found to have uniformly decreased (16867 cells/hive for C; 14153 cells/hive for T and 13933 cells/hive for R). This observation can be explained by the unfavourable weather conditions before set-up, followed by the confined conditions inside the tunnel tents.

After one honeybee brood cycle, i.e. 3 weeks after the 3rd test item application (fourth colony assessment, DAA21), the mean abundance of brood had increased to 28893 cells/hive for C, 29700 cells/hive for T and 26253 cells/hive for R. Within the time period up to the fifth colony assessment on DAA28, the mean number of brood cells increased in C to 36007 and in T to 30287 cells/hive. In the reference item group R, the mean number of brood cells decreased during the same period of time to 24713 cells/hive.

The brood development was comparable between the control and the test item treatment colonies, the differences were within the range of natural variation. No test item related effects on brood development were observed.

Development of the Food Storage Area

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The mean extent of food stores in the colonies (sum of cells containing nectar and pollen) assessed before set-up of the hives (first colony assessment) was 11953 cells/hive for C, 14681 cells/hive for T and 17013 cells/hive for R. At the second colony assessment, at the end of the exposure period on DAA7, the mean extent of food stores in C, T and R was found to have decreased to 11000 cells/hive for C, 12100 cells/hive for T and 11440 cells/hive for R. It was noticed, that all colonies in C, T and R had no pollen stores at the end of the confined exposure conditions (except of colony 1R with only 220 pollen cells). This can be explained by the restricted foraging area during the confinement in the tunnel tents (DAA-4 to7), which obviously did not allow for the storage of excess *Phacelia*-pollen, concurrently to the ongoing feeding of the brood.

At the subsequent colony assessment, after the end of the confined exposure period outside the tunnels on DAA14 (third colony assessment), the mean extent of food stores in the colonies C, T and R increased to 16427 cells/hive for C, 18920 cells/hive for T and 18407 cells/hive for R. At the two following colony assessments (fourth and fifth colony assessment, DAA21 and DAA28), the mean extent of food stores in the colonies in C and T increased further and remained on a comparable level (DAA21: 27207 cells/hive for C and 24860 cells/hive for T; DAA28: 26987 cells/hive for C and 25667 cells/hive for T).

The observed decrease in food stores in both, treatment and control, during confinement as well as the subsequent increase can be considered as typical for this type of study. No test item related adverse effects on the development of the food storage area were observed.

Honeybee Behaviour

In the control group C, unusual behaviour was observed on the day of application approximately one hour after tap water treatment (approximately 100 bees sitting at the hive entrance, some cleaning, some shivering). On the following two days and on DAA5 and 6, clustering at the hive entrance was observed (alternately in 1C, 2C and 3C) and once aggressiveness at hive 1C. In the test item treatment group T a slight repellent effect became obvious by an imbalance between an intensive flying activity and an infrequent landing on the crop (DAA0aa). Also clustering at the hive entrance was observed at hive 2T. On the following day, intensive flying over the crop coupled with an infrequent landing on the crop was still observed for colony 1T and 3T, clustering at the hive entrance at colony 2T and 3T. In the reference item treatment group R, clear symptoms of intoxication were observed on the day of application, after the treatment (2R); during the following days, clustering at the hive entrance (DAA1; 1R, 2R) and intensive flight activity coupled with a considerably reduced frequency of landing on the crop (DAA5; 1R) was described.

Since clustering at the hive entrance was observed in all treatment groups it did not happen in relation to the test item application

Residue Analysis

Details of the analytical results as obtained by analysing samples of flowers of *Phacelia tanacetifolia,* nectar and wax as well as soil samples are given in an Analytical Phase Report, which is attached to the report.

C. Validity criteria

The test was considered valid because a detectable effect of the reference item was found (e. g. high adult bee mortality).

CONCLUSION

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A soil application of the test item, corresponding to 300 g BYI 02960 a.i./ha, followed by immediate soil incorporation and the subsequent sowing of *Phacelia*-seeds was performed in combination with a pre-flowering foliar application of the test item, corresponding to 200 g BYI 02960 a.i./ha, followed by a further foliar application of the test item, corresponding to 200 g BYI 02960 a.i./ha during full-flowering of a highly bee-attractive crop (*Phacelia tanacetifolia*) with honeybees actively foraging on the crop.

This application scenario did not cause treatment-related adverse effects on mortality, brood and food development as well as on colony vitality throughout the entire observation time. A slight repellent effect of the test item was observed on the day of the 3rd test item application, after the treatment.

Report:	**KIIIA1 10.4.7/06; [redacted] S. (2012)**
Title:	Determination of Side-Effects of BYI 02960 SL 200 G on Honey Bee (*Apis mellifera* L.) Brood Under Confined Semi-Field Conditions
Report No:	S10-03819
Document No:	M-427438-01-1
Guidelines:	**OECD Guidance Document No. 75 (2007)**
Deviations:	**None**
GLP:	**Yes (certified laboratory)** **Air temperature, relative air humidity and daily precipitation were non-GLP records.**

Executive summary

The effect of BYI 02960 SL 200 G was tested on the honeybee (*Apis mellifera* L.) under confined semi-field conditions following the OECD guidance document No. 75 (2007): Guidance Document on the Honey Bee (*Apis mellifera* L.) Brood Test under Semi-Field Conditions, with modifications.

The aim of the study was to evaluate potential effects of spray applications of BYI 02960 SL 200 G (Batch No: 2010-007173; Sample description: FAR01535-00; Specification No: 102000021884-01; Analysed content of a.i. BYI 02960: 16.9% w/w, 198.6 g/L) on the honeybee, *Apis mellifera carnica* L. as well as the residues resulting from the application in bee products at the end of the study. The crop used for this semi-field study was *Phacelia tanacetifolia*, the study was conducted in [redacted], federal state of Baden-Württemberg, Germany.

This study included three treatment groups with three replicates (tunnels) each: one tap-water control group (C), one test-item group (T) and one reference item group (R). In the test item treatment group, the crop was sprayed 5 days before set-up of the hives in the tunnels at BBCH 59-61 (end of inflorescence emergence, flower buds visible, but still closed, up to 10% flowers open; 1st test item application) and five days after set-up at BBCH 63-65 (full-flowering; 2nd test item application), during honeybees actively foraging on the crop under confined conditions; the target application rate of the test item corresponded at the first and second application to 200 g BYI 02960 a.i./ha, respectively.

Concurrently to the 2nd test item application, tap water was applied in the control group and Insegar® 25 WG was applied at a target rate of 600 g product/ha in the reference item group (corresponding to 150 g fenoxycarb per ha). The spray volume per application was 200 L/ha in the test item treatment T and 400 L/ha in the reference item and control treatment, respectively. The colony size at set-up was in the range of 5313 – 8750 bees. The honeybees remained 12 days in the tunnels.

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The first colony assessment was performed before set-up of the colonies in the tunnel tents. Subsequently, six further colony assessments were conducted. Overall, the colonies were assessed once before, twice during and four times after the end of the confined exposure phase. Mortality assessments (in bee trap and on the linen sheets) started 4 days before the 2nd test item application and continued on a daily basis for 7 days after the 2nd test item application. Further mortality assessments were conducted at the monitoring site, after the end of the confinement period, only in the bee traps until DAA27. Flight intensity assessments started four days before the 2nd test item application and continued on a daily basis until the 7th day after the 2nd test item application. Residue samples (pollen, nectar and wax from inside the hives) were taken in the test item treatment group at the end of the study on DAA27.

The analytical phase was conducted at Bayer CropScience AG, [redacted], Germany.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

Overall conclusion:

A pre-flowering foliar application of the test item, corresponding to 200 g BYI 02960 a.i./ha to a highly bee-attractive crop (*Phacelia tanacetifolia*), was followed by a further foliar application of the test item, again corresponding to 200 g BYI 02960 a.i./ha, during full-flowering with honeybees actively foraging on the crop.

With particular respect to bee brood development, as quantitatively assessed via digital image analysis of individual cells, the tested BYI 02960 application scenario has not caused adverse effects on the survival of marked eggs (brood termination rate), on brood development from eggs into adult bees (brood index) as well as on the brood compensation ability (brood compensation index).

Overall, the employed application scenario did not cause treatment-related adverse effects on mortality, on flight intensity, on honeybee behaviour, on brood- and food development as well as on colony vitality under forced exposure conditions.

A slight repellent effect of the test item was indicated by a reduced flight intensity on the day of the 2nd test item application as well as on some further days during the confined exposure period.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	BYI 02960 SL 200 G
Specification number:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear, orange-brown liquid
Batch No.:	2010-007173
Sample description:	FAR01535-00
Material number:	79718845
Nominal content of active substance:	BYI 02960: 200 g/L
Analytical content of active substance:	BYI 02960: 16.9% w/w; 198.6 g/L acc. to certificate of analysis
Density:	1.175 g/mL at 20°C
Stability of test compound:	Expiry date: 30.09.2012, when stored at 25 ± 5°C in original container in the dark (also acceptable from +2 to +30°C)

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Control: Untreated water control (400 L/ha)

Reference Item:

The information concerning the reference item according to the substance container label and data sheet:

Name:	Insegar 25 WG
Manufacturer:	[redacted]
Batch No.:	SMO8D313
Nominal content of active substance:	Fenoxycarb: 250 g/kg
Analytical content of active substance:	Not available
Type of formulation:	WG
Chemical state and description:	Solid, grey brown granules
Density:	Not applicable
Stability:	To be stored at cold (+ 4 °C)

2. Test organisms

Species:	*Apis mellifera*
Common name:	Honey bee
Source:	Small, healthy honey bee colonies with 10 combs from one breeding line (Colony size: 5313 –8750 bees / colony at set-up

B. Study design and methods

1. In life dates: July 02 to August 11, 2011

2. Experimental treatments

The semi-field test was located in [redacted], federal state of Baden-Württemberg, Germany and was conducted in tunnels located on a field of flowering *Phacelia tanacetifolia*, a surrogate crop specifically recommended in the OECD guidance document No. 75 (2007) for this kind of study.

This study included three treatment groups with three replicates (tunnels) each: one tap-water control group (C), one test-item group (T) and one reference item group (R). In the test item treatment group, the crop was sprayed 5 days before set-up of the hives in the tunnels at BBCH 59-61 (end of inflorescence emergence, flower buds visible, but still closed, up to 10% flowers open; 1st test item application) and five days after set-up at BBCH 63-65 (full-flowering; 2nd test item application), during honeybees actively foraging on the crop under confined conditions; the target application rate of the test item corresponded at the first and second application to 200 g BYI 02960 a.i./ha, respectively.

Concurrently to the 2nd test item application, tap water was applied in the control group and Insegar® 25 WG was applied at a target rate of 600 g product/ha in the reference item group (corresponding to 150 g fenoxycarb per ha). The spray volume per application was 200 L/ha in the test item treatment T and 400 L/ha in the reference item and control treatment, respectively.

The individual treatment groups, the respective application rates per ha and the respective spray volumes per ha are described in the table below.

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Table 10.4.7- 9: Treatment groups, application rates and spray volume

Treatment Group	Code	Time of application	Target application rate [g a.i./ha]*	[g product/ha]	Spray Volume
Control (tap water)	C	DAA0, BBCH 63-65	None	None	400 L/ha
Test item (BYI 02960 SL 200 G)	T	DAA-10, BBCH 59-61	200	1 L	200 L/ha
		DAA0, BBCH 63-65	200	1 L	200 L/ha
Reference item (Insegar® 25 WG)	R	DAA0, BBCH 63-65	150	600 g	400 L/ha

DAA = days after 2nd test item application
* calculation based on the nominal content of a.i.

3. Observations and measurements

The first colony assessment was performed before set-up of the colonies in the tunnel tents. Subsequently, six further colony assessments were conducted. Overall, the colonies were assessed once before, twice during and four times after the end of the confined exposure phase. Mortality assessments (in bee trap and on the linen sheets) started 4 days before the 2nd test item application and continued on a daily basis for 7 days after the 2nd test item application. Further mortality assessments were conducted at the monitoring site, after the end of the confinement period, only in the bee traps until DAA27. Flight intensity assessments started four days before the 2nd test item application and continued on a daily basis until the 7th day after the 2nd test item application. Residue samples (pollen, nectar and wax from inside the hives) were taken in the test item treatment group at the end of the study on DAA27.

The analytical phase was conducted at Bayer CropScience AG, [redacted], Germany.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Total and mean number of dead bees on the linen sheets in tunnel tents and in the dead bee traps;
- Flight intensity (mean number of forager bees/m2 and treatment group on P. tanacetifolia before as well as after the 2nd application in T and the concurrent applications in C and R, respectively);
- Behaviour of the bees in the crop and around the hive;
- Condition of the colonies and development of the bee brood (number of bees (strength), mean values of the different brood stages per colony and assessment date);
- Development of the bee brood assessed in individual brood cells.

RESULTS AND DISCUSSION

A. Analytical Findings

Details of the analytical results as obtained by analysing samples of pollen, nectar and wax, as collected in the test item treatment group at the end of the study at DAA27, are given in an Analytical Phase Report, which is attached to the report

B. Biological Findings

Honeybee Mortality

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After set-up of the colonies inside the tunnels until the day of the 2nd test item application (DAA-4 to 0ba), the mean mortality value was 50.5, 86.8, and 93.7 dead bees/day for the treatment group C, T, and R, respectively.

On the day of the 2nd test item application, immediately before the 2nd test item application in T and the concurrent (1st and only) application in C and R, respectively (DAA0ba), the mean mortality value was 71.0, 165.0, and 125.0 dead bees/day for the treatment group C, T, and R, respectively.

On the day of the 2nd test item application, after the 2nd test item application in T and the concurrent (1st and only) application in C and R, respectively (DAA0aa), the mean mortality decreased in all treatment groups to values of 46.3, 91.3, and 43.7 dead bees/day for the treatment group C, T, and R, respectively.

The daily mortality values during the confined exposure period after the 2nd test item application in T and the concurrent application in C and R, respectively (DAA0aa to DAA7), were comparable in all treatment groups with mean values of 71.0, 91.0 and 96.5 dead honey bees in C, T and R, respectively, and were as such well in line with the mean mortality values of 50.5, 86.8, and 93.7 dead bees/day in C, T, and R, respectively, before the 2nd test item application (DAA-4 to 0ba). The slightly higher, although not statistically significantly different mean mortality in the test item treatment group T when compared to control before the 2nd test item application (DAA-4 to 0ba), can be explained by natural, colony-based variability, as mortality in T was lower than in R, which was during this period of time as untreated as C.

During the confined exposure period after the 2nd test item application and the concurrent application in C and R, respectively (DAA0aa to DAA7), the daily mortality was in a range from 34.0 to 160.7 dead honey bees in C, 31.3 to 151.3 dead honey bees in T and 30.7 to 177.3 dead honey bees in R, and as such virtually identical in all treatment groups. None of these values in T and R were statistically significantly higher than the corresponding value in the control.

During the further monitoring of the colonies outside the tunnels at a remote monitoring location (DAA8 to DAA27), daily mortality was in a range from 8.0 to 109.3 dead bees in C, 6.7 to 81.7 dead bees in T and 2.3 to 96.0 dead bees in R. Only on two single days, DAA9 and DAA21, the mortality of the test item treatment was statistically significantly higher compared to the control.

In the total time period after the 2nd test item application and the concurrent application in C and R, respectively (DAA0aa to 27), the mean daily mortality was comparable in C, T and R and was recorded to be 44.9, 51.3, and 45.3 for C, T, and R, respectively. Neither the mean mortality values before the 2nd test item application nor the mean mortality values after the 2nd test item application and the concurrent application in C and R, respectively, were statistically significantly different, when comparing the performance in the test item treatment group and in the reference item treatment group with the performance of the control group (t-test, method pooled, one-sided for the test item treatment and post-application period of the reference item treatment, two-sided for the pre-application period of the reference item treatment; $\alpha = 0.05$).

During the daily assessments of mortality (DAA0aa to 27), the sum of dead pupae, dead young bees, dead malformed bees and dead malformed pupae found inside the dead bee traps was lowest in the test item treatment T, when compared to the control and reference item treatment (18 in C, 4 in T and 40 in in R).

When comparing the mean mortality before application (DAA-4 to DAA0ba) until the day of the 2nd test item application, the $Q_{M(DAA0aa)}$ values were calculated to be 0.9, 1.1 and 0.5 for the treatment groups C, T and R, respectively. The $Q_{M(DAA0aa\ to\ 7)}$ values were calculated to be 1.4, 1.0 and 1.0 for C, T and R, respectively.

Overall, it can be concluded that BYI 02960, when applied according to the application scenario as outlined above, comprising an application corresponding to 200 g BYI 02960 a.i./ha during full-flowering of a highly bee-attractive crop (*Phacelia tanacetifolia*) with honey bees actively foraging on the crop under forced exposure conditions, does not cause treatment-related adverse effects on mortality.

Table 10.4.7- 10: Toxicity to Honey Bees, Semi-Field Test Under Confined Exposure Conditions

Test item	BYI 02960 SL 200 G		
Test object	*Apis mellifera*		
Start of confining honeybee colonies in tunnel tents	T: 5 days after 1st test item application, 5 days before 2nd test item application (12 days in tunnel) C: 5 days before application (12 days in tunnel) R: 5 days before application (12 days in tunnel)		
Treatment group	Control (C)	BYI 02960 SL 200 G (T)	Reference item (R)
Application rate	--	2 × 200 g BYI 02960 a.i./ha at BBCH 59-61 and 63-65, respectively	1 × 600 g Insegar 25 WG/ha at BBCH 63-65
Mean mortality DAA-4 to 0ba [dead bees/day]	50.5	86.8	93.7
Mean mortality DAA0aa [dead bees/day]	46.3	91.3	43.7
Mean mortality DAA0aa to 7 [dead bees/day]	71.0	91.0	96.5
Mean mortality DAA0aa to 27 [dead bees/day]	44.9	51.3	45.3
Daily mean flight intensity DAA-4 to 0ba [bees/m²/min]	16.3	14.5	15.4
Daily mean flight intensity DAA0aa [bees/m²/min]	14.6	8.8	6.7
Daily mean flight intensity DAA1 [bees/m²/min]	24.6	25.9	25.8
Daily mean flight intensity DAA0aa to 7 [bees/m²/min]	15.1	11.7	10.3

DAA = Days after 2nd test item application
ba = before application
aa = after application

Honeybee Flight Intensity

After set-up of the colonies inside the tunnels until the day of the 2nd test item application and the concurrent application in C and R, respectively (DAA-4 to 0ba), the mean daily flight intensity was 16.3, 14.5, and 15.4 honeybees/m² in C, T, and R, respectively. The daily flight intensity during this period was in a range from 6.0 to 22.3 forager bees/m² in C, 5.0 to 20.7 in T and 7.0 to 19.3 in the R, respectively.

On the day of the 2nd test item application (DAA0aa), after application, the mean daily flight intensity across 7 assessments within a period of about 6 hours was recorded to be 14.6, 9.2, and 6.7 honeybees/m², for C, T, and R, respectively, and was statistically significantly reduced in T and R, when compared to C (t-test, method pooled, one-sided, $\alpha = 0.05$). One day after the 2nd test item application and the concurrent application in C and R, respectively (DAA1), the mean flight intensity was increased in all treatment groups compared to the pre-application period and was almost on an identical level with 24.6, 25.9 and 25.8 honeybees/m² in the C, T and R, respectively.

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During the confined exposure period after the 2nd test item application and the concurrent application in C and R, respectively (DAA0aa to DAA7), the flight intensity in the test item treatment was also statistically significantly reduced on DAA4 and DAA6 and in the reference item on DAA3, 4 and 6, when compared to control (t-test, method pooled, one-sided, $\alpha = 0.05$). The daily flight intensity during this period was in a range from 0.0 to 27.7 forager bees/m² in C, 0.0 to 25.9 in T and 0.0 to 25.8 in R, respectively. During the same time period (DAA0aa to 7), the mean daily flight intensity was recorded to be 15.1, 11.8 and 10.3 for C, T and R, respectively, and was statistically lower in the test item treatment and in the reference item treatment when compared to control (t-test, method pooled, one-sided, $\alpha = 0.05$). Although being statistically significantly different from control on DAA0aa, 4, 6 and during DAA0aa to DAA7 (mean), the values of 9.2, 17.7, 16.0 and 11.8 (mean) forager bees/m² in T are not biologically relevant, since foraging activity was overall on a still high level.

Overall, a slight repellent effect of the test item was indicated by a reduced flight intensity on the day of the 2nd test item application (DAA0aa) as well as on some further days during the confined exposure period.

Behaviour of the bees

No abnormal behaviour was recorded in the control, in the test item and in the reference item treatment before the 2nd test item application and the concurrent application in C and R, respectively (DAA-4 to DAA0ba).

In the test item treatment T after the 2nd test item application on DAA0aa, a few bees were observed with intoxication symptoms (cramping, locomotion problems, coordination problems), moreover, an intensive flying over the crop coupled with an infrequent landing on the crop could be observed together with a slight repellent effect. On the following days (DAA1 to 7), normal behaviour was observed in all three tunnels of the test item treatment.

In the reference item treatment R, after application on DAA0aa, a strong repellent effect (no or low flight activity on the crop) as well as several bees with obvious signs of intoxication were observed.

In the control group, normal behaviour was recorded throughout the observation period except for the evening of DAA0aa, were about 200 to 500 bees were observed motionless on the covering net in C3 as well as in T1 – T3, respectively. In the test item treatment group, during the last assessment in the evening of DAA0aa (+6h), no bees were found motionless on the covering net any more.

Except for DAA0aa, honey bee behaviour in test item treatment T was comparable to the control treatment throughout the entire assessment period.

Development of Honey Bee Brood in Individual Cells (Digital Image Analysis)

According to the development time of a worker honey bee from egg to adult bee (imago), which normally averages to 21±1 days, it can be expected that young bees will have hatched until the assessment date BFD+22 (i.e. 22 days after the **B**rood Area **F**ixing **D**ay).

The control (C) and treatment (T) colonies showed a successful development, with rising brood indices over the entire assessment period, except for the assessment on BFD15, where stable values (due to the long development time of the sealed brood) or a slight reduction compared to the previous assessment on BFD11 were observed in both, C and T, respectively.

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In the reference item treatment (R), the brood index decreased at the first assessment after application (on BFD5) and remained on a low level throughout the further assessments. In total, the brood indices were 1.00 in each treatment at the first BFD assessment and reached at the last assessment on BFD22 mean values of 3.98 in C, 4.34 in T and 0.26 in R, respectively.

Table 10.4.7- 11: Summary of the brood and compensation indices and termination rates

Replicate	Brood / Compensation indices at x days after brood area fixing day (BFD)					Termination rate (BFD22)
	0	+5	+11	+15	+22	[%]
C1	1.00 / 1.00	2.40 / 2.40	3.16 / 3.16	3.12 / 3.12	3.90 / 4.25	22.05
C2	1.00 / 1.00	2.68 / 2.71	3.38 / 3.45	3.34 / 3.45	4.18 / 4.53	16.39
C3	1.00 / 1.00	2.36 / 2.37	3.19 / 3.22	3.10 / 3.16	3.87 / 4.36	22.57
Mean C	**1.00 / 1.00**	**2.48 / 2.49**	**3.24 / 3.28**	**3.19 / 3.24**	**3.98 / 4.38**	**20.34**
STD	0.00 / 0.00	0.17 / 0.19	0.12 / 0.15	0.13 / 0.18	0.17 / 0.14	3.43
T1	1.00 / 1.00	2.57 / 2.57	3.48 / 3.52	3.48 / 3.52	4.36 / 4.37	12.88
T2	1.00 / 1.00	2.31 / 2.34	3.47 / 3.56	3.47 / 3.57	4.34 / 4.51	13.27
T3	1.00 / 1.00	2.54 / 2.57	3.48 / 3.58	3.46 / 3.57	4.33 / 4.63	13.48
Mean T	**1.00 / 1.00**	**2.47 / 2.49**	**3.48 / 3.55**	**3.47 / 3.55**	**4.34 / 4.50**	**13.21**
STD	0.00 / 0.00	0.14 / 0.13	0.01 / 0.03	0.01 / 0.03	0.02 / 0.13	0.30
R1	1.00 / 1.00	0.33 / 0.33	0.18 / 0.20	0.16 / 0.17	0.20 / 0.21	95.98
R2	1.00 / 1.00	0.47 / 0.84	0.26 / 0.45	0.26 / 0.49	0.33 / 1.23	93.42
R3	1.00 / 1.00	0.19 / 0.71	0.19 / 1.27	0.19 / 1.40	0.24 / 2.33	95.30
Mean R	**1.00 / 1.00**	**0.33 / 0.63**	**0.21 / 0.64**	**0.20 / 0.69**	**0.26 / 1.26**	**94.90**
STD	0.00 / 0.00	0.14 / 0.27	0.04 / 0.56	0.05 / 0.64	0.07 / 1.06	1.33

The compensation indices in C and T were comparable with their respective corresponding brood indices. Both indices showed nearly the same course. At BFD5, i.e. the first assessment after the 2nd test item application and the concurrent application in C and R, respectively, the compensation index was with 2.49 identical in C and T, respectively, and almost identical at BFD22; this virtually identical mean compensation index of 4.38 in the control and of 4.50 in T at BFD22 indicates a comparable new egg laying activity in those few cells that had been emptied before successful hatch. In contrast, in the reference item treatment R, the compensation index decreased to 0.63 at BFD5 and increased only slightly thereafter to a value of 1.26 at BFD22.

At the last assessment (BFD22), the termination rate was 20.34% in the control and 13.21% in T, compared to a value of 94.90% in the reference item treatment R.

Overall, the quantitative assessments of brood development in individually marked cells revealed that the application scenario as outlined above, comprising an application corresponding to 200 g BYI 02960 a.i./ha during full-flowering of a highly bee-attractive crop (*Phacelia tanacetifolia*), with honey bees actively foraging on the crop under forced exposure conditions, does not cause treatment related adverse effects on honey bee brood development.

Strength of the Colonies

The mean number of bees assessed before set-up of the hives (first colony assessment, DAA-7/-5) in the tunnels revealed a comparable colony strength in all treatment groups with an average of 7354 bees/hive in C [range: 6125 - 8750], 7542 bees/hive in T [range: 7250 – 8125], and 7125 bees/hive in R [range: 5313 – 8125].

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At the third colony assessment on DAA4 (during the confined exposure period), the mean number of bees in C and T was still comparable and remained almost on the same level as at first colony assessment, whereas the mean number of bees in the reference item group R was reduced (C: 7521, T: 7625, R: 5938).

At the subsequent colony assessment, after the end of the confined exposure period outside the tunnels on the remote monitoring location (DAA10, fourth colony assessment), the mean number of bees had increased in all treatment groups and was again on a comparable level (C: 9209, T: 8792, R: 8959).

At the following colony assessments the mean number of bees in C and T increased and decreased in parallel (DAA14: C = 8021, T = 8229; DAA21: C = 8896, T = 8750; DAA27: C = 7813, T = 8000), whereas on the same assessment days, the mean number of bees in R was found to be reduced to 7896 on DAA14, to 7459 on DAA21 and to 6292 on DAA27.

The development of colony strength was comparable between C and T throughout the study period and showed the fluctuations which are typical of this endpoint. As such, no test-item related adverse effects on colony strength were observed.

Development of Brood

The mean abundance of brood (sum of cells containing eggs, larvae, and pupae) assessed before set-up of the hives (first colony assessment, DAA-7/-5) was comparable in all treatment groups with 20400 cells/hive for C ,19667 cells/hive for T and 19600 cells/hive for R. At the second colony assessment (DAA-1), the mean abundance of brood in C, T and R had decreased slightly (19267 cells/hive for C, 17867 cells/hive for T and 19533 cells/hive for R).

At the third colony assessment, during the confined exposure period, on DAA4, the mean abundance of brood in C and T was on an almost identical level (C: 17533 cells/hive, T: 17933 cells/hive), whereas the mean abundance of brood in R decreased strongly to 13400 cells/hive.

On the fifth colony assessment (DAA14), no appreciable changes were observed in the mean abundance of brood in C and T, whereas a strong decrease in R to 8333 cells/hive occurred. This refers to a clearly detectable effect of the reference item, which is typical for this point in time.

Brood of all stages (eggs, larvae, capped brood) was present in all colonies at all assessments during the study, with the exception of colony T1, which was found queenless from the fourth to the last colony assessment. The number of brood cells consequently decreased from this time on (fifth - seventh colony assessment) in colony T1 and as such, from the fifth colony assessment onwards, also the mean values in T were lower than in C (sixth colony assessment, DAA21: 20200 cells/hive for C, 13667 cells/hive for T, seventh colony assessment, DAA27: 18600 cells/hive for C, 12667 cells/hive for T).

Except for the colony T1, the fluctuations of all brood stages were within the range of natural variation and typical for this kind of study.

Overall, honey bee brood development and colony conditions in test item treatment T were comparable to control treatment during the whole assessment period. No test-item related adverse effects on brood development were observed.

Development of the Food Storage Area

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The mean extent of food stores in the colonies (sum of cells containing nectar and pollen) assessed before set-up of the hives (first colony assessment, DAA-5) was 14067 cells/hive for C, 17333 cells/hive for T and 14600 cells/hive for R. At the second colony assessment (DAA-1), the mean extent of food stores decrease slightly in C and increased slightly in T and R (C: 13067 cells/hive , T: 17600 cells/hive, R: 15733 cells/hive). At the third colony assessment, during the confined exposure period, on DAA4, the mean extent of food stores in the colonies C, T and R had decreased (C: 9067 cells/hive , T: 16333 cells/hive, R: 11933 cells/hive). At the two subsequent assessments on the remote monitoring location at DAA10 and DAA14 (fourth and fifth colony assessment), the mean extent of food stores in C and T had decreased in parallel, increased thereafter in parallel on DAA21 and decreased again in parallel to 4400 cells/hive in C and 5133 cells/hive in T at DAA27. The mean extent of food stores in the R hives from DAA10 to DAA27 was an alternate de- and increase with 6133 cells/hive on the last colony assessment.

The observed parallel decrease in food stores in both, treatment and control, during confinement as well as the subsequent alternate, but again parallel de- and increase in C and T, respectively, can be considered as typical for this type of study. No test-item related adverse effects on the development of the food storage area were observed.

Residue Analysis

Details of the analytical results as obtained by analysing samples of pollen, nectar and wax, as collected in the test item treatment group at the end of the study at DAA27, are given in an Analytical Phase Report, which is attached to the study report.

C. Validity criteria

The study is considered valid since the expected effects in the reference item group actually occurred.

CONCLUSION

A pre-flowering foliar application of the test item, corresponding to 200 g BYI 02960 a.i./ha to a highly bee-attractive crop (*Phacelia tanacetifolia*), was followed by a further foliar application of the test item, again corresponding to 200 g BYI 02960 a.i./ha, during full-flowering with honeybees actively foraging on the crop.

With particular respect to bee brood development, as quantitatively assessed via digital image analysis of individual cells, the tested BYI 02960 application scenario has not caused adverse effects on the survival of marked eggs (brood termination rate), on brood development from eggs into adult bees (brood index) as well as on the brood compensation ability (brood compensation index).

Overall, the employed application scenario did not cause treatment-related adverse effects on mortality, on flight intensity, on honeybee behaviour, on brood- and food development as well as on colony vitality under forced exposure conditions.

A slight repellent effect of the test item was indicated by a reduced flight intensity on the day of the 2nd test item application as well as on some further days during the confined exposure period.

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IIIA1 10.5 Effects on arthropods other than bees

Toxicity tests on non-target arthropods have been performed with BYI 02960 SL 200 G on the species *Typhlodromus pyri*, *Aphidius rhopalosiphi*, *Coccinella septempunctata*, *Aleochara bilineata* and *Orius laevigatus*. Furthermore, two full-fauna off-crop field studies were conducted.

A summary of the results is provided in Table 10.5- 1.

Table 10.5- 1: Ecotoxicological endpoints for arthropods other than bees (BYI 02960 SL 200)

Test species, Dossier-File-No. Reference	Tested Formulation, Study type, Exposure	Ecotoxicological Endpoint
Aphidius rhopalosiphi [redacted], 2010 M-366965-01-2 KIIA 8.8.1.1/01 KIIIA1 10.5.1/01	BYI 02960 SL 200 (g/L) Laboratory, glass plates 0.5 g a.i./ha 1.1 g a.i./ha 2.2 g a.i./ha 4.7 g a.i./ha 10 g a.i./ha 10 g a.i./ha 20 g a.i./ha 40 g a.i./ha 80 g a.i./ha 160 g a.i./ha	LR_{50} < 0.5 g a.i./ha Corr. Mortality [%] 85 100 100 100 100 100 100 100 100 100
Typhlodromus pyri [redacted], 2010 M-366957-01-2 KIIA 8.8.1.2/01 KIIIA 10.5.1/02	BYI 02960 SL 200 (g/L) Laboratory, glass plates 2 g a.i./ha 4 g a.i./ha 9 g a.i./ha 19 g a.i./ha 40 g a.i./ha	LR_{50} 17.3 g a.i./ha Corr. Mortality [%] 6.3 6.3 25.0 50.0 89.6
Aphidius rhopalosiphi [redacted], 2010 M-366970-01-2 KIIIA1 10.5.2/01	BYI 02960 SL 200 (g/L) Extended lab., exposure on potted barley seedlings 0.5 g a.i./ha 0.89 g a.i./ha 1.58 g a.i./ha 2.81 g a.i./ha 5.0 g a.i./ha	LR_{50} 2.02 g a.i./ha Corr. Mortality [%] / Effect on Reproduction [%] / Repellency rel. to control [%] -3.4 [A] / -6.4 [B] / -42.0 [C] sign. 10.3 / -2.9 [B] / -45.8 [C] n.sign. 37.9 / 57.7 / -26.3 [C] n.sign. 69.0 / n.a. / -67.5 [C] sign. 89.7 / n.a. / -43.7 [C] sign.
Typhlodromus pyri [redacted], 2010 M-366968-01-2 KIIIA1 10.5.2/02	BYI 02960 SL 200 (g/L) Extended lab., exposure on detached broad bean leaves 15 g a.i./ha 32 g a.i./ha 67 g a.i./ha 142 g a.i./ha 300 g a.i./ha	LR_{50} 177 g a.i./ha Corr. Mortality [%] / Effect on Reproduction [%] -1.1 [A] / 6.0 -4.6 [A] / 0.6 6.9 / 5.1 37.9 / -6.8 [B] 75.9 / n.a.

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Test species, Dossier-File-No. Reference	Tested Formulation, Study type, Exposure	Ecotoxicological Endpoint
Coccinella	BYI 02960 SL 200 (g/L)	LR_{50} 273.9 g a.i./ha
septempunctata	Extended lab., exposure on	
[redacted], 2010	detached broad bean leaves	Corr. Mortality [%] / Eggs/Female/Day / Hatching [%]
M-384754-01-2	Control	- / 10.4 / 92.0
KIIIA1 10.5.2/03	8 g a.i./ha	12.9 / 15.5 / 91.4
	17 g a.i./ha	4.1 / 17.6 / 95.7
	35 g a.i./ha	-9.7 [A] / 9.5 / 87.3
	72 g a.i./ha	3.2 / 15.5 / 93.5
	150 g a.i./ha	32.3 / 21.6 / 95.4
	Control	- / 13.9 / 91.9
	100 g a.i./ha	9.7 / 16.1 / 92.0
	160 g a.i./ha	19.4 / 21.0 / 92.3
	250 g a.i./ha	45.2 / 25.6 / 91.3
	380 g a.i./ha	71.0 / n.a. / n.a.
	600 g a.i./ha	96.8 / n.a. / n.a.
Aleochara bilineata	BYI 02960 SL 200 (g/L)	ER_{50} > 300 g a.i./ha
[redacted], 2010	Extended lab., spray deposits	
M-384433-01-2	on soil (LUFA 2.1)	Effect on Reproduction [%]
KIIIA1 10.5.2/04	10 g a.i./ha	7.6
	21 g a.i./ha	18.2
	45 g a.i./ha	0.4 [B]
	95 g a.i./ha	13.2
	200 g a.i./ha	16.5
	300 g a.i./ha	13.0
Aphidius rhopalosiphi	BYI 02960 SL 200 (g/L)	
[redacted], 2010	Aged residue spray deposits	
M-396372-01-2	on maize plants, 2 appl. of	
KIIIA1 10.5.3/01	250 g a.i./ha (spray interval of 10 d)	Corr. Mortality [%] / Effect on Reproduction [%] / Repellency rel. to control [%]
	Residues aged for 0 d:	100 / n.a. / 68.1 sign.
	Residues aged for 14 d:	90.0 / n.a. / 47.5 sign.
	Residues aged for 28 d:	76.7 / n.a. / 30.6 sign.
	Residues aged for 42 d:	29.6 / 89.9 / 37.3 n.sign.
	Residues aged for 49 d:	20.0 / 37.6 / -18.8 [C] n.sign.
	Residues aged for 56 d:	6.7 / 0.6 / -6.0 [C] n.sign.
Orius laevigatus	BYI 02960 SL 200 (g/L)	
[redacted], 2010	Aged residue spray deposits	
M-394033-01-2	on apple plants, 2 appl. of	Corr. Mortality [%] / Effect on Fecundity [%] / Effect on Fertility [%]
KIIIA1 10.5.3/02	250 g a.i./ha (spray interval of 10 d)	
	Residues aged for 0 d:	100 / n.a. / n.a.
	Residues aged for 14 d:	75.6 / n.a. / n.a.
	Residues aged for 28 d:	24.5 / 23.0 / -6.5 [D]
	Residues aged for 42 d:	9.8 / 34.7 / 12.9
NTA off-crop field study (Netherlands)	BYI 02960 SL 200 (g/L)	Community level NOER = 21 g a.i./ha
[redacted] & [redacted], 2012	NTA full fauna off-crop field study, Spray application rates:	Population level NOER = 5.1 g a.i./ha
M-425092-01-2	0.51, 1.7, 5.1, 21 g a.i./ha	Population level NOEAER = 21 g a.i./ha
KIIIA1 10.5.4/01		NOER: No Observed Effect Rate
		NOEAER: No Observed Ecologically Adverse Effect Rate

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Test species, Dossier-File-No. Reference	Tested Formulation, Study type, Exposure	Ecotoxicological Endpoint
NTA off-crop field study (South-West France) [redacted] & [redacted], 2012 M-425080-01-2 KIIIA1 10.5.4/02	BYI 02960 SL 200 (g/L) NTA full fauna off-crop field study. Spray application rates: 0.51, 1.7, 5.1, 21 g a.i./ha	Community level NOER = 21 g a.i./ha Population level NOER = 1.7 g a.i./ha Population level NOEAER = 21 g a.i./ha NOER: No Observed Effect Rate NOEAER: No Observed Ecologically Adverse Effect Rate

A: A negative value indicates a lower mortality in the treatment than in the control.
B: A negative value indicates a higher reproduction rate in the treatment than in the control.
C: A negative value indicates a higher percentage of wasps found on plants in the treatment than in the control.
D: A negative value indicates a higher nymphal hatching rate in the treatment than in the control.
n.a.: Not assessed.
sign.: statistically significant at 5%-level.
n.sign.: not statistically significant.

The tier 1 glass plate studies indicate that insects, as represented by *Aphidius rhopalosiphi* (LR_{50} < 0.5 g a.i./ha), are more sensitive to the exposure of BYI 02960 SL 200 G than mites like *Typhlodromus pyri* (LR_{50} 17.3 g a.i./ha). This has been confirmed by extended laboratory studies for the same two species. A comparison of the extended laboratory results for *Aphidius rhopalosiphi* (LR_{50} 2.02 g a.i./ha, ER_{50} >0.89 g a.i./ha) with the results from the additionally tested species *Coccinella septempunctata* (LR_{50} 273.9 g a.i./ha and no effects on reproduction below the LR_{50}) and *Aleochara bilineata* (ER_{50} >300 g a.i./ha) gives clear evidence, that *Aphidius rhopalosiphi* is by far the most sensitive species.

Hence, aged residue studies were conducted with *Aphidius rhopalosiphi* and - in addition - with the predatory bug *Orius laevigatus*. The results showed that *Orius laevigatus* is also susceptible to the exposure of BYI 02960 SL 200 G (effects <50% after 28 days of aging) but clearly less sensitive than *Aphidius rhopalosiphi* (effects <50% after 49 days of aging). For this reason, the risk assessment of *Orius laevigatus* is considered to be covered by the risk assessment for *Aphidius rhopalosiphi*.

Risk assessment procedures

The risk assessment was performed according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) and to the Guidance Document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (ESCORT 2, [redacted] et al. 2000[11]).

Tier 1 risk assessment

In-field hazard quotient (HQ)

The following equation was used to calculate the hazard quotient (HQ) for the in-field scenario:

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

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In field-HQ = max. single application rate * MAF / LR_{50}

The risk is considered acceptable if the calculated HQ is < 2.

BYI 02960 SL 200 G is intended to be applied in the field with an application rate of 1 x 150 g a.i./ha in hops (BBCH 31-75) and an application rate of 1 x 125 g a.i./ha in lettuce (BBCH 12 - 49). Therefore, the multiple application factor for both uses (MAF) was set at 1.0.

Resulting HQ values are presented in Table 10.5- 2.

Table 10.5- 2: HQ for terrestrial non-target arthropods for the in-field scenario

Crop (field uses)	Species	Appl. rate [g a.i./ha]	MAF	LR_{50} [g a.i./ha]	HQ	Trigger	Refined risk assessment required
Hops	*A. rhopalosiphi*	150	1	<0.5	300	2	yes
Hops	*T. pyri*	150	1	17.3	8.7	2	yes
Lettuce	*A. rhopalosiphi*	125	1	<0.5	250	2	yes
Lettuce	*T. pyri*	125	1	17.3	7.2	2	yes

Conclusion: For the standard species, the in-field HQ values are above the trigger of concern, indicating a need for refinement.

Off-field hazard quotient (HQ)

The following equation was used to calculate the hazard quotient (HQ) for the off-field scenario:

Off-field HQ = max. single application rate * MAF * (drift factor/VDF)*correction factor / LR_{50}

- Max. single application rate = 125 g a.i./ha (lettuce), 150 g a.i./ha (hops)
- MAF (multiple application factor) = 1 (only 1 application)
- Drift factor = 19.33% (hop, 3 m distance, 1 application; ESCORT2) 2.77% (lettuce (field crops), 1m distance, 1 application; ESCORT2)
- VDF (vegetation distribution factor) = 10 (default value as recommended by the Terrestrial Guidance Document, to take into account the 3-dimensional structure of the off-field vegetation)
- Correction factor = 10 (uncertainty factor for the extrapolation from indicator species to all off-field non-target arthropods; default value for tier 1 risk assessment according to the Terrestrial Guidance Document)

The risk is considered acceptable if the calculated HQ is < 2.

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Table 10.5- 3: HQ for terrestrial non-target arthropods for the off-field scenario

Crop	Species	Appl. rate [g a.i./ha]	MAF	Drift [%]	VDF	Corr. factor	LR_{50} [g a.i./ha]	HQ	Trigger
Hops	*A. rhopalosiphi*	150	1	19.33	10	10	<0.5	>58.0	2
Hops	*T. pyri*	150	1	19.33	10	10	17.3	1.7	2
Lettuce	*A. rhopalosiphi*	125	1	2.77	10	10	<0.5	>6.9	2
Lettuce	*T. pyri*	125	1	2.77	10	10	17.3	0.2	2

Conclusion: The HQ values for *Typhlodromus pyri* are below the trigger of concern for both uses (hops and lettuce). However, both HQ values for *Aphidius* are above the trigger of 2, indicating a need for refinement.

Tier 2 risk assessment

Potential exposure

The exposure scenario is based on the use pattern as given in Table 10-1. The product is applied once at a rate of 150 g a.i./ha in hops and once at a rate of 125 g a.i./ha in lettuce.

According to ESCORT2 and the Terrestrial Guidance Document the exposure is calculated as:

In-field: Application rate * MAF

In-field (high crops) Application rate * MAF * 0.5 [12]

Off-field: Application rate * MAF * (drift factor / VDF) * correction factor

- Application rates: 150 g a.i./ha (hops)
  125 g a.i./ha (lettuce)
- Drift factor: 19.33% (hop, 3 m distance, 1 application; ESCORT2)
  2.77% (lettuce (field crops), 1 m distance, 1 application; ESCORT2)
- MAF (multiple application factor) = 1 (default value for 1 application).
- VDF (vegetation distribution factor) = 10 (default value as recommended by the Terrestrial Guidance Document, to take into account the 3-dimensional structure of the off-field vegetation; in can only be applied in the context of 2D test systems)
- Correction factor = 5 (uncertainty factor for the extrapolation from indicator species to all off-field non-target arthropods; default value for tier 2 risk assessment according to the Terrestrial Guidance Document)

[12] Correction factor of 0.5 for the in-field exposure assessment in high crops according to ESCORT2 (see footnote "a" in legend on page 19 of the ESCORT2 document)

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Table 10.5- 4: Exposure calculation for in-field assessment

Crop / no. of applications	Appl. rate [g a.i./ha]	MAF	in-field $PEC_{max.}$ [g/ha]
Hops / 1	150	1	75
Lettuce / 1	125	1	125

Table 10.5- 5: Corrected exposure for off-field risk assessment

Appl. rate [g a.i./ha]	MAF	Drift [%]	Veg. distr. factor	Correction factor	off-field $PEC_{max.}$ [g/ha]	Remark
150	1	19.33	-	5	144.9	in case of 3-D study design
150	1	19.33	10	5	14.5	in case of 2-D study design
125	1	2.77	-	5	17.3	in case of 3-D study design
125	1	2.77	10	5	1.73	in case of 2-D study design

Tier 2 in-field risk assessment

Table 10.5- 6: In-field risk assessment based on study results from extended laboratory studies

Test Species	in-field PEC_{max} [g a.i./ha]	LR_{50}/ ER_{50} [g a.i./ha]	Trigger	Refined assessment required?
Use in hops, 1 x 150 g a.i./ha				
Typhlodromus pyri	75	177	Effects are < 50%	no
Aphidius rhopalosiphi	75	0.89	Effects are < 50%	**yes**
Coccinella septempunctata	75	273.9	Effects are < 50%	no
Aleochara bilineata	75	>300	Effects are < 50%	no
Use in lettuce, 1 x 125 g a.i./ha				
Typhlodromus pyri	125	177	Effects are < 50%	no
Aphidius rhopalosiphi	125	0.89	Effects are < 50%	**yes**
Coccinella septempunctata	125	273.9	Effects are < 50%	no
Aleochara bilineata	125	>300	Effects are < 50%	no

The higher tier in-field risk assessment for *Typhlodromus*, *Coccinella* and *Aleochara* indicates that no unacceptable adverse effects are to be expected in the in-field area for arthropod species with a similar sensitivity as these species. However, the in-field risk assessment for *Aphidius rhopalosiphi* indicates that initial effects in the in-field area cannot be excluded. Therefore, further refinement is needed.

Refined in-field risk assessment

The results of the tier 2 risk assessment indicated that initial effects on species with a similar sensitivity as *Aphidius rhopalosiphi* cannot be excluded. As a consequence, an aged residue study was performed to demonstrate the potential for recovery for *Aphidius rhopalosiphi*, the most sensitive tested species and in addition for the predatory bug *Orius laevigatus*.

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An extended laboratory aged residue study has been performed on *Aphidius rhopalosiphi* ([redacted], 2010; M-396372-01-2, KIIIA1 10.5.3/01). In this study, BYI 02960 SL 200 was applied 2 times at a rate of 250 g a.i./ha with a 10 days interval on potted maize plants. Spray residues were aged under semi-field conditions. Bioassays with freshly dried residues, residues aged for 14 days and for 28 days, respectively, resulted in a corrected morality of 100, 90 and 76.7%, respectively. After an aging time of 42, 49 and 56 days, corrected mortalities of 29.6, 20.0 and 6.7% were recorded, respectively. Exposure to residues aged for 42 days resulted in a 89.9% reduction in reproduction relative to the control. No effects on reproduction >50% were observed after an aging time of 49 and 56 days, respectively. Statistically significant repellency was recorded in the first, second and third bioassay, while in the fourth, fifth and sixth bioassay no repellent effects were observed.

A second extended laboratory aged residue study has been performed on *Orius laevigatus* ([redacted], 2010; M-394033-01-2, KIIIA1 10.5.3/02). BYI 02960 SL 200 was applied 2 times at a rate of 250 g a.i./ha with an application interval of 10 days onto potted apple plants. *Orius laevigatus* showed a corrected mortality of 100 and 75.6%, when exposed to fresh residues of the test item and residues aged for 14 days, respectively. After an aging time of 28 and 42 days, a corrected mortality of 24.5 and 9.8% was observed, respectively. No adverse effects on reproduction > 50% were observed after an aging time of 28 and 42 days, respectively. The results indicated that *Orius laevigatus* is as well susceptible to the exposure of BYI 02960 SL 200 G (effects <50% after 28 days of aging) but clearly less sensitive as *Aphidius rhopalosiphi* (effects <50% after 49 days of aging).

These aged residue studies indicate that the potential for recovery is given even after 2 applications at a rate of 250 g a.i./ha within 7 weeks for the most sensitive species, *Aphidius rhopalosiphi*. For *Orius laevigatus,* residues aged for only 4 weeks already had no adverse effect on mortality and reproduction. Since the intended use pattern includes only single applications at a rate of up to 150 g a.i./ha in the field it can be concluded that the potential for recovery is given within a few weeks after the application and no unacceptable in-field risk for non-target arthropods has to be expected from the use of BYI 02960 SL 200 according to the proposed use pattern.

Tier 2 off-field risk assessment

Table 10.5- 7: Off-field risk assessment based on study results from extended laboratory studies

Test Species	off-field PEC_{max} [g a.i./ha]	LR_{50}/ ER_{50} [g a.i./ha]	Trigger	Refined assessment required?
Use in hops, 1 x 150 g a.i./ha				
Typhlodromus pyri	14.5	177	Effects are < 50%	no
Aphidius rhopalosiphi	144.9	0.89	Effects are < 50%	**yes**
Coccinella septempunctata	14.5	273.9	Effects are < 50%	no
Aleochara bilineata	14.5	>300	Effects are < 50%	no
Use in lettuce, 1 x 125 g a.i./ha				
Typhlodromus pyri	1.73	177	Effects are < 50%	no
Aphidius rhopalosiphi	17.3	0.89	Effects are < 50%	**yes**
Coccinella septempunctata	1.73	273.9	Effects are < 50%	no
Aleochara bilineata	1.73	>300	Effects are < 50%	no

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The maximum PEC off-field for the use in hop (worst-case) is calculated to be 14.5 g/ha for 2D-test systems and 144.9 g/ha for 3D-test systems. For *Typhlodromus*, *Coccinella and Aleochara* no effects > 50% neither on mortality nor on reproduction were observed in extended laboratory studies on natural substrate at a rate of 142 (*Typhlodromus*), 250 (*Coccinella*, 2nd trial) and 300 g a.i./ha (*Aleochara*), respectively (see Table 10.5-1). However, for *Aphidius*, initial effects cannot be excluded, indicating a need for further refinement.

Refined off-field risk assessment

To assess off-field effects of BYI 02960 SL 200 on naturally occurring arthropod communities under more realistic conditions, two full-fauna field studies were conducted on grassland as surrogate for off-field habitats in the Netherlands and in Southwestern France.

BYI 02960 SL 200 was applied in a dose-response design at drift rates (0.51, 1.7, 5.1 and 21 g a.i./ha) to grassland habitats with little agricultural input in the Netherlands and Southwestern France. These sites held a diverse and representative off-crop non-target arthropod community. Four replicate plots of 22 x 22 m each were used per treatment (4 application rates, control, reference treatment = 24 plots in total). Arthropods were sampled comprehensively using three different sampling methods (pitfall, suction and weed/Berlese sampling) shortly before the application and 1, 2, 4 and 8 weeks after the application. Overall community changes relative to the control were analyzed using multivariate statistics and depicted by Principal Response Curves (PRC).
In addition, effects on individual arthropod populations were analyzed with univariate statistics for taxa that were sufficiently abundant. In both studies, the toxic reference item treatment caused clear responses both at the arthropod community level and at the population level, demonstrating that the test system was sufficiently sensitive to detect toxic effects.

Results of the trial in the Netherlands:

At community level, no statistically significant effects on arthropod communities were found at any of the rates tested up to and including 21 g a.i./ha. Therefore, 21 g a.i./ha is the community NOER (No Observed Effect Rate) of BYI 02960 SL 200. In addition, 72 taxa were sufficiently abundant for a univariate statistical evaluation at population level. In the three lowest test rates, none of the taxa showed a consistent treatment-related response. Predatory mites of the family Cunaxidae and hymenopteran parasitoids of the family Braconidae showed transient adverse effects only at one sampling moment shortly after application of the highest test rate (21 g a.i./ha). Both taxa recovered within one week. In conclusion, the population NOEAER (No Observed Ecologically Adverse Effect Rate) of BYI 02960 SL 200 is 21 g a.i./ha and the population NOER (No Observed Effect Rate) is 5.1 g a.i./ha.

Results of the trial in France:

At community level, no statistically significant effects on arthropod communities were found at any of the rates tested up to and including 21 g a.i./ha. Therefore, 21 g a.i./ha is the community NOER (No Observed Effect Rate) of BYI 02960 SL 200.

At the population level, 79 taxa were sufficiently abundant for population level evaluations. Only three phytophagous taxa were adversely affected.

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At the test rate of 5.1 g a.i./ha, significant adverse effects on the chrysomelid beetles Alticinae and on juvenile leafhoppers (Cicadellidae) occurred at a single sampling occasion. At the rate of 21 g a.i./ha, Alticinae and Cicadellidae showed statistically significant reductions at two sampling moments after application, but recovered already 4 weeks after the application. Aphids (Aphidoidea) were reduced at several sampling moments after the application of 21 g a.i./ha; however, the reduction was only statistically significant at the penultimate sampling moment. The observed effects on Aphidoidea were to be expected since aphids are a target pest species for the proposed uses of BYI 02960 SL 200 in lettuce and hop. However, aphids can be expected to recover quickly from population declines due to their high asexual reproductive potential. No significant reduction in Aphidoidea abundance was observed at the last sampling moment two months after application of the test item. The highest rate tested in this study - BYI 02960 SL 200 applied at 21 g a.i./ha - is therefore classified as the population NOEAER (No Observed Ecologically Adverse Effect Rate).

Refined potential exposure:

As a wide range of species naturally occurring in off-field habitats has been tested in the two full-fauna field studies, the default correction factor of 5 for the off-field PEC calculations (addressing the uncertainty concerning the sensitivity of off-field arthropod species) can therefore be reduced to 1.

$Off\text{-}field_{PEC\ refined}$: Application rate * MAF * drift factor *correction factor

Table 10.5- 8: Refined exposure for off-field risk assessment

Crop / no. of applications	Appl. rate [g a.i./ha]	MAF	Drift [%]	Correction factor*	off-field $PEC_{max.}$ [g/ha]	Remark
Hops / 1	150	1	19.33	1	14.5	for full-fauna field study
Lettuce / 1	125	1	2.77	1	3.5	for full-fauna field study

*C.F. reduced to 1 due to testing of a wide range of species in two full-fauna field studies

The results of the two field studies demonstrate that an exposure to BYI 02960 SL 200 at 21 g a.i./ha does not adversely affect arthropod communities in off-field habitats (community NOER in both studies 21 g a.i./ha). The taxa which were statistically significantly reduced at the highest tested rate of 21 g a.i./ha all recovered within 4 to 8 weeks after the application (population NOEAER in both studies 21 g a.i./ha). As the maximum off-field PEC lies below 21 g a.i./ha for the proposed use patterns in hops and lettuce, no unacceptable adverse effects on non-target arthropods are to be expected in the off-field area.

IIIA1 10.5.1 Effects on sensitive species already tested, artificial substrates

Laboratory tests on artificial substrate (glass plates) have been conducted with the BYI 02960 SL 200 on the standard species *Aphidius rhopalosiphi* and *Typhlodromus pyri.* The summaries are presented in the Annex II document (see IIA 8.8.1). However, a short overview is given below.

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Report:	**KIIIA1 10.5.1/01; [redacted] (2010)**
Title:	Toxicity to the parasitoid wasp *Aphidius rhopalosiphi* (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) using a laboratory test; BYI 02960 SL 200 (g/L)
Report No:	CW09/079
Document No:	M-366965-01-2
Guidelines:	[redacted] **ET AL. (2000),** [redacted] **ET AL. (2001)**
Deviations:	**None**
GLP:	**Yes (certified laboratory)**

Executive Summary

The objective of this laboratory study was to investigate the lethal and sublethal toxicity of BYI 02960 SL 200 (Sample description: FAR01438-00 (Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 102000021884)) on the parasitoid wasp *Aphidius rhopalosiphi* 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 (4 replicates 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 a toxic reference item. The study had to be done 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, 2.2, 4.7 and 10 g a.i./ha 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 LR_{50} was calculated to be <0.5 g a.i./ha.

Due to the still high mortality in the second trial, no assessment of reproductive capacity was performed.

Report:	**KIIIA1 10.5.1/02; [redacted] (2010)**
Title:	Toxicity to the predatory mite *Typhlodromus pyri* SCHEUTEN (Acari, Phytoseiidae) using a laboratory test; BYI 02960 SL 200 g/L
Report No:	CW09/073
Document No:	M-366957-01-2
Guidelines:	[redacted] **ET AL. (2000);** [redacted] **ET AL. (2001)**
Deviations:	**None**
GLP:	**Yes (certified laboratory)**

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Executive Summary

The test item BYI 02960 SL 200 (Sample description: FAR01438-00 (Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 102000021884)) was tested under laboratory conditions after residual contact exposure of protonymphs of the predatory mite *Typhlodromus pyri* to spray residues with rates of 2, 4, 9, 19 and 40 g a.i./ha in 200 L deionized water/ha applied on glass plates. The control was treated with deionized water (200 L/ha). Dimethoate EC 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 dead mites. The number of escaped mites was calculated as the difference from the total number exposed.

At the test item rates of 2 and 4 g a.i./ha a corrected mortality of 6.3% each was observed. At the higher rates of 9, 19 and 40 g a.i./ha, a corrected mortality of 25.0, 50.0 and 89.6%, respectively, occurred.

The LR_{50} was calculated to be 17 g a.i./ha. (95% CI: 13 to 21 g a.i./ha).

IIIA1 10.5.2 Effects on non-target terrestrial arthropods in ext. laboratory tests

Report:	**KIIIA1 10.5.2/01; [redacted], (2010)**
Title:	Toxicity to the parasitoid wasp *Aphidius rhopalosiphi* (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) using an extended laboratory test on barley; BYI 02960 SL 200 (g/L)
Report No:	CW09/083
Document No:	M-366970-01-2
Guidelines:	[redacted] **ET AL. (2000),** [redacted] **ET AL. (draft 2006),** [redacted] **ET AL. (2001)**
Deviations:	**During the mortality phase the humidity decreased twice (for the duration of 3 hours and 5 hours) to 55%. This is not considered to have had any impact on the test results.**
GLP:	**Yes (certified laboratory)**

Executive Summary

The objective of this extended laboratory study was to investigate the lethal and sublethal toxicity of BYI 02960 SL 200 (Sample description: FAR01438-00 (Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 102000021884)) to the parasitoid wasp *Aphidius rhopalosiphi* when exposed on a plant surface.

The test item was applied on barley seedlings at rates of 0.5, 0.89, 1.58, 2.81 and 5.0 g a.i./ha and effects on *Aphidius rhopalosiphi* were compared to those of a deionised water treated control. A toxic reference (active substance: dimethoate) applied at 3.0 g a.i./ha was included to indicate the relative susceptibility of the test organisms and the test system.

Mortality of 30 females (6 replicates with 5 wasps per test group) was assessed 2, 24 and 48h after exposure. Repellency of the test item was assessed during the initial 3 h after the release of the females. Five separate observations were made at 30-minute intervals starting 15 minutes after the introduction of all wasps. From the water control and the dose rates 0.5, 0.89 and 1.58 g a.i./ha, 15 impartially chosen females per treatment were each transferred to a cylinder containing untreated barley seedlings infested with *Rhopalosiphum padi* for a period of 24 h. The number of mummies was assessed 11 days later.

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At the dose rate of 0.5 g a.i./ha, no mortality was detected. In the rates of 0.89 and 1.58 g a.i./ha. 10.3 and 37.9% corrected mortality was observed, respectively. 69.0 and 89.7% corrected mortality were found in the 2.81 and 5.0 g a.i./ha rates, respectively. No repellent effect of the test item was observed. No reduction in reproductive success relative to the control was observed in the 0.5 and 0.89 g a.i./ha rates. A reduction of 57.7% was detected at the 1.58 g a.i./ha rate.

The LR_{50} was calculated to be 2.02 g a.i./ha. (95% CI: 1.61 to 2.55 g a.i./ha).

MATERIAL AND METHODS

A. Materials

1. Test material

Test item:	BYI 02960 SL 200
Specification No.:	102000021884
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Material number:	79718845
Sample description:	FAR01438-00
Batch No.	2009-001253
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.0% w/w, 199.8 g/L according to certificate of analysis
Density:	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.: 414.8 g/L)

3. Test organism

Species:	*Aphidius rhopalosiphi*
Common name:	Parasitoid wasp
Age:	48 h
Source of test organism:	*Aphidius rhopalosiphi* used for testing were supplied [redacted]
Source of host organism:	The barley seedlings were provided by the horticultural group of BCS Global Biology Herbicides. *Rhopalosiphum padi* (aphids used for parasitation in the reproduction assessment) were taken from the breeding of the testing facility.

B. Study design and methods

1. In life dates   January 11 to February 16, 2010

2. Design of the test

Number of test groups:	7 (control, test and reference item)
Number of application rates:	Test item: 5 Reference item: 1
Number of replicates per test group:	6 (one replicate = one exposure unit (pot with barley seedlings))
Number of larvae/per replicate:	5

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The test item (soluble concentrate formulation of BYI 02960 SL 200 (g/L)) was applied at rates of 0.5, 0.89, 1.58, 2.81 and 5.0 g a.i./ha, respectively, on barley seedlings and the effects on the parasitoid wasp *Aphidius rhopalosiphi* were compared to those of a deionised water treated control. A toxic reference (active substance: dimethoate) applied at 3.0 g a.i./ha was included to indicate the relative susceptibility of the test organisms and the test system.

After 48 h, 15 impartially chosen females per treatment from the water control and the dose rates 0.5, 0.89 and 1.58 g a.i /ha were each transferred to a cylinder containing untreated barley seedlings infested with *Rhopalosiphum padi* for a period of 24 h to assess reproduction.

3. Observation and measurements

Mortality was assessed by recording the condition of the test animals 2, 24 and 48 h after application:

- live (alive and apparently unaffected)
- affected (showing reduced co-ordination or any abnormal behaviour)
- moribund (unable to walk, but still moving legs or antennae)
- dead (no longer moving)

Repellency of test item was assessed by five separate observations at 30-minute intervals starting 15 minutes after the introduction of all wasps.

Reproduction was assessed by counting the number of mummies 11 days after the transfer of female wasps to cylinders.

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 and repellency data were tested for normal distribution using the Shapiro-Wilk test and for homogeneity using the Levene test.

As the repellency data in this study were normally distributed and homogenous one-way ANOVA and the Williams test (one-sided; $\alpha = 0.05$) were used.

As the reproduction data in this study were not normally distributed the Wilcoxon test (one-sided with Bonferroni-Holm adjustment; $\alpha = 0.05$) was used.

The LR_{50} value was calculated using Probit analysis.

RESULTS AND DISCUSSION

A. Environmental Conditions

Wasps were kept under conditions which are summarized as follows:

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Test temperature:	19.0-22.0°C
Relative humidity:	60 - 78 % (deviations: decrease for 3h and 5 h to 55% during mortality phase).
Photoperiod:	16 hours light / 8 hours dark
Light source	523 - 955 Lux (mortality phase) 981 – 3240 Lux (parasitation phase) 4590 - 19410 Lux (reproduction phase)

B. Biological Findings

A summary of effects of BYI 02960 SL 200 on mortality and reproduction of *Aphidius rhopalosiphi* exposed on barley seedlings is given below:

Table 10.5.2- 1 Effects of BYI 02960 SL 200 (g/L) on mortality and reproduction of *Aphidius rhopalosiphi*

Test item		BYI 02960 SL 200 (g/L)					
Test organism		*Aphidius rhopalosiphi*					
Exposure on:		Barley seedlings					
		Mortality after 48 hours [%]			Reproduction		
Treatment	**g a.i./ha**	**Uncorr.**	**Corr.**	**P-Value(*)**	**Rate (mummies/female)**	**Red. rel. to Control [%]**	**P-Value(#)**
Control	0	3.3			23.0		
Test item	0.5	0	-3.4	1.000 n.sign.	24.5	-6.4	0.803 n.sign.
Test item	0.89	13.3	10.3	0.353 n.sign.	23.7	-2.9	0.458 n.sign.
Test item	1.58	40.0	37.9	0.002 sign.	9.7	57.7	0.012 sign.
Test item	2.81	70.0	69.0	<0.001 sign.	n.a.	n.a.	
Test item	5.0	90.0	89.7	<0.001 sign.	n.a.	n.a.	
Reference item	3.0	100	100		n.a.	n.a.	

LR_{50}: 2.02 g a.i./ha; 95% Confidence Interval: (1.61 – 2.55) (calculated with Probit analysis)
* Fisher's Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm
# Wilcoxon test (one-sided), p-values are adjusted according to Bonferroni-Holm
n.a. not assessed
n.sign. not significant
sign. Significant

Mortality

After 48 h of the study 3.3% of the wasps were found dead in the control group. All wasps survived in the 0.5 g a.i./ha rate. In the group treated with 0.89 g a.i./ha, 10.3% of the wasps were dead.

A statistically significant corrected mortality was found in the 1.58, 2.81 and 5.0 g a.i./ha rates with 37.9%, 69.0% and 89.7%, respectively. In the reference item group, all wasps were dead after 48 h of exposure.

Repellency

During the observations in the initial 3 h of the test a mean of 35.3% of the wasps settled on the plants in the control group. In the groups treated with 0.5, 2.81 and 5.0 g a.i./ha, respectively, a significantly larger percentage of wasps settled on the plants. No significant difference to the control was found in the groups treated with 0.89 and 1.58 g a.i./ha. In the toxic reference group 58.2% of the wasps were found on the plants. Thus, no repellent effect was observed for the test item.

Table 10.5.2- 2: Repellency of BYI 02960 SL 200 (g/L) to *Typhlodromus pyri*

Repellency (comparison per mean values)				
Treatment	**g a.i./ha**	**% Wasps on plant**	**Red. rel. to Control [%]**	**P-Value (#)**
Control	0	35.3		
Test item	0.5	50.2	-42	0.045 sign.
Test item	0.89	51.5	-45.8	0.052 n.sign.
Test item	1.58	44.6	-26.3	0.055 n.sign.
Test item	2.81	59.2	-67.5	0.014 sign.
Test item	5.0	50.8	-43.7	0.014 sign.
Reference item	3.0	58.2	-64.6	

one-way ANOVA, Williams test (one-sided)
n.sign. not significant
sign. Significant

Reproduction

The mean number of mummies per female in the control group was 23.0. This compared to 24.5 mummies/female in the 0.5 g a.i./ha rate of the test item, 23.7 mummies/female in the 0.89 g a.i./ha rate and 9.7 mummies/female in the 1.58 g a.i./ha rate of BYI 02960 SL 200.

No reduction (-6.4 % and -2.9 %) in reproductive success relative to the control occurred in the 0.5 and 0.89 g a.i./ha rate. A statistically significant reduction of 57.7% was detected at the 1.58 g a.i./ha rate.

C. Validity Criteria

The validity criteria for the extended laboratory test ([redacted] ET AL., 2006) of mortality ≤10%, an average number of ≥5 mummies per female and no more than 2 wasps producing no mummies in the control group and ≥50% corrected mortality in the toxic reference are fulfilled.

D. Biological Endpoints Derived

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

LR_{50}: 2.02 g a.i./ha (95% Confidence Interval: 1.61 – 2.55)

CONCLUSION

The effects of BYI 02960 SL 200 residues on the survival of the parasitoid wasp *Aphidius rhopalosiphi* can be quantified as an LR_{50} of 2.02 g a.i./ha (95% CI: 1.61 to 2.55 g a.i./ha). No repellent effect of the test item was observed. No reduction in reproductive success relative to the control was observed in the 0.5 and 0.89 g a.i./ha rate, respectively. A reduction of 57.7% was detected at the 1.58 g a.i./ha rate.

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Report:	**KIIIA1 10.5.2/02; [redacted]. (2010)**
Title:	Toxicity to the predatory mite *Typhlodromus pyri* [redacted] (Acari, Phytoseiidae) using an extended laboratory test on *Phaseolus vulgaris*; BYI 02960 SL 200 (g/L)
Report No:	CW09/076
Document No:	M-366968-01-2
Guidelines:	**[redacted] ET AL. (2000) modified: Use of natural substrate (detached bean leaves) instead of glass plate; [redacted] ET AL. (2001)**
GLP:	**Yes (certified laboratory)**

Executive Summary

The objective of this extended laboratory study was to investigate the lethal and sub lethal toxicity of BYI 02960 SL 200 (Sample description: FAR01438-00; Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 102000021884) applied onto detached leaves to the predatory mite *Typhlodromus pyri*.

The test item was applied to detached leaves of *Phaseolus vulgaris* at rates of 15, 32, 67, 142 and 300 g a.i./ha and the effects on *Typhlodromus pyri* were compared to those of a deionised water treated control. A toxic reference (active substance: dimethoate) applied at 40 g a.i./ha was included to indicate the relative susceptibility of the test organisms and the test system. Mortality of 100 mites (5 replicates with 20 individuals per test group) was assessed 1, 4, 7, 10, 12 and 14 days after exposure by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed. The reproduction rate of surviving mites was then evaluated from day 7 to day 14 days after treatment by counting the total number of offspring (eggs and larvae) produced.

At the test rates of 15 and 32 g a.i./ha, no corrected mortality was detected. At the test rates of 67, 142 and 300 g a.i./ha, a corrected mortality of 6.9, 37.9 and 75.9% has been observed, respectively.

The LR_{50} was calculated to be 177 g a.i./ha (95% CI: 151 to 205 g a.i./ha). At 15, 32, 67 and 142 g a.i./ha the reproduction was reduced by 6.0, 0.6, 5.1 and -6.8%, respectively.

MATERIAL AND METHODS

A. Materials

1. Test material

Test item:	BYI 02960 SL 200
Specification No.:	102000021884
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Material number:	79718845
Sample description:	FAR01438-00
Batch No.:	2009-001253
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.0% w/w, 199.8 g/L, according to certificate of analysis
Density:	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

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Solvent:	No solvent used; deionized water was used as diluent for the test item and for the reference item
Reference substance:	Dimethoate EC 400 (analytical content of a.i.: 428.5 g/L)

3. Test organism

Species:	*Typhlodromus pyri*
Common name:	Predatory mite
Age:	Protonymphs
Source of test organism:	[redacted]
Source of host organism:	The plants (*Phaseolus vulgaris*) were provided by the horticultural group of BCS Global Biology Herbicides

B. Study design and methods

1. In life dates — January 14 to February 18, 2010

2. Design of the test

Number of test groups:	7 (control, test and reference item)
Number of application rates:	Test item: 5 Reference item: 1
Number of replicates per test group:	5 (one replicate = one exposure unit (leaf disc))
Number of larvae/per replicate:	20

The test item (soluble concentrate formulation of BYI 02960 SL 200 (g/L)) was applied to detached leaves of *Phaseolus vulgaris* at rates of 15, 32, 67, 142 and 300 g a.i./ha, respectively, and the effects on the predatory mite *Typhlodromus pyri* were compared to those of a deionised water treated control. A toxic reference (active substance: dimethoate) applied at 40 g a.i /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 replicates with 20 individuals per test group) was assessed 1, 4, 7, 10, 12 and 14 days, respectively, 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.

The reproduction rate of surviving mites was then evaluated from day 7 to day 14 days after treatment by counting the total number of offspring (eggs and larvae) produced.

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.

As the reproduction data in this study were not normally distributed the Wilcoxon test (one-sided with Bonferroni-Holm adjustment; $\alpha = 0.05$) was used.

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The LR_{50} value was calculated using Probit analysis.

RESULTS AND DISCUSSION

A. Environmental Conditions

Mites were kept under conditions which are summarized as follows:

Test temperature:	25.0-26.0°C
Relative humidity:	65 - 70% (deviations < 2 h down to 43%)
Photoperiod:	16 hours light / 8 hours dark
Light source	638 - 1217 Lux

B. Biological Findings

The mortality / escaping rate in the control exposure units up to day 7 after treatment was 13.0%. The mean corrected mortality of the nymphs, and the mean reproduction rate of the surviving females exposed to the test item and the toxic reference is given below:

Table 10.5.2- 3: Effects of BYI 02960 SL 200 (g/L) on mortality and reproduction of *Typhlodromus pyri*

Test item		BYI 02960 SL 200 (g/L)					
Test organism		*Typhlodromus pyri*					
Exposure on:		Detached bean leaves					
		Mortality after 7 days [%]			Reproduction		
Treatment	g a.i./ha	Uncorr.	Corr.	P-Value(*)	Rate (eggs per female)	Red. rel. to Control [%]	P-Value (#)
Control	0	13.0			4.5		
Test item	15	12.0	-1.1	1.000 n.sign.	4.3	6.0	1.000 n.sign.
Test item	32	9.0	-4.6	1.000 n.sign.	4.5	0.6	1.000 n.sign.
Test item	67	19.0	6.9	0.502 n.sign.	4.3	5.1	1.000 n.sign.
Test item	142	46.0	37.9	<0.001 sign.	4.8	-6.8	1.000 n.sign.
Test item	300	79.0	75.9	<0.001 sign.	n.d.	n.d.	
Reference item	40	100	100		n.d.	n.d.	

LR_{50}: 177 g a.i./ha; 95 % Confidence Interval: (151 - 205) (calculated with Probit analysis)
* Fisher`s Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm
# Wilcoxon test (one-sided), p-values are adjusted according to Bonferroni-Holm
n.d. not detected n.sign. not significant sign. significant

C. Validity Criteria

The validity criteria of mortality ≤ 20 % in the control group, ≥ 50% corrected mortality in the toxic reference and an average number of ≥ 4 eggs per female are fulfilled (laboratory method with glass plates ([redacted] ET AL., 2000)).

D. Biological Endpoints Derived

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

LR_{50}: 177 g a.i./ha (95 % Confidence Interval: 151 - 205 g a.i./ha)

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CONCLUSION

The effects of BYI 02960 SL 200 residues on the survival of the predatory mite *Typhlodromus pyri* under extended laboratory conditions can be quantified as an LR_{50} of 177 g a.i./ha (95% CI: 151 to 205 g a.i./ha).

Report:	**KIIIA1 10.5.2/03; [redacted] (2010)**
Title:	Toxicity to the ladybird beetle *Coccinella septempunctata* L. (Coleoptera, Coccinellidae) using an extended laboratory test on *Phaseolus vulgaris*: BYI 02960 SL 200 (g/L)
Report No:	CW09/074
Document No:	M-384754-01-2
Guidelines:	[redacted] ET AL. (2000) MODIFIED: USE OF NATURAL SUBSTRATE (BEAN LEAVES) INSTEAD OF GLASS PLATE;; [redacted] ET AL. (2001)
Deviations from guideline:	**None**
GLP:	**Yes (certified laboratory)**

Executive Summary

The objective of this extended laboratory study was to investigate the lethal and sublethal toxicity of BYI 02960 SL 200 (first trial: Sample description: FAR01438-00 (Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 102000021884); second trial: Sample description: TOX 08854-00 (Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 102000021884-01)) to the ladybird beetle *Coccinella septempunctata* when exposed to treated leaf surfaces.

The test item was applied onto detached bean leaves (*Phaseolus vulgaris*) at rates of 8, 17, 35, 72 and 150 g a.i./ha in a first trial and dose rates of 100, 160, 250, 380 and 600 g a.i./ha in a second trial. Effects on preimaginal survival of 40 larvae per treatment of *Coccinella septempunctata* were assessed until the hatch of the imagines (up to up to 13 days in the first and 17 days in the second trial). A toxic reference (active substance: dimethoate) applied at 12 g a.i./ha was included to indicate the relative susceptibility of the test organisms and the test system. The control was treated with deionized water (200 L/ha). Reproduction of the surviving hatched adults was assessed for the 8, 17, 35, 72, 100, 150, 160 and 250 g a.i./ha rates of BYI 02960 SL 200 over a period of 17 days.

The dose rates of 8, 17, 35, 72, 100, 150 and 160 g a.i./ha had no influence on preimaginal mortality. At the higher test item rates of 250, 380 and 600 g a.i./ha, a corrected preimaginal mortality of 45.2, 71.0 and 96.8%, respectively, could be observed. The LR_{50} was calculated to be 273.9 g a.i./ha. (95% CI: 185.9 to 330.8 g a.i./ha). Because the reproductive performance was within the historical data base for control beetles (≥ 2 fertile eggs per female and day, [redacted] ET AL. 2000) this parameter is considered as not affected at all these test item rates.

MATERIAL and Methods

A. Materials

1. First trial

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1. Test material

Test item:	BYI 02960 SL 200
Specification No.:	102000021884
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear dark brown liquid
Material number:	79718845
Sample description:	FAR 01438-00
Batch No.:	2009-001253
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 199.8 g/L (17.0% w/w) according to certificate of analysis
Density:	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
Negative control:	Deionized water
Positive control:	Reference substance: Dimethoate EC 400 (analysed content: 428.5 g a.i./L)

3. Test organism

Species:	*Coccinella septempunctata*
Common name:	Ladybird beetle
Age:	4 days
Source of test organism:	Lady bird eggs were supplied [redacted]
Source of host organism:	The plants (*Phaseolus vulgaris*) were provided by the horticultural group of BCS Global Biology Herbicides

2. Second trial

1. Test material

Test item:	BYI 02960 SL 200
Specification No.:	102000021884-01
Material number:	79718845
Sample description:	TOX 08854-00
Batch No.:	2009-001253
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.0% w/w, 199.8 g/L according to certificate of analysis
Density:	1.174 g/mL
Stability of test compound	Expiry date: 02.12.2010 (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
Negative control	deionized water
Positive control:	Reference substance: Dimethoate EC 400 (analysed content: 414.8 g a.i./L)

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3. Test organism

Species:	*Coccinella septempunctata*
Common name:	Ladybird beetle
Age:	4 days
Source of test organism:	Lady bird eggs were supplied [redacted]
Source of host organism:	The plants (*Phaseolus vulgaris*) were provided by the horticultural group of BCS Global Biology Herbicides

B. Study design and methods

1. In life dates November 12, 2009 to April 23, 2010

2. Design of the test

Number of test groups:	7 (control, test and reference item) per test trial
Number of application rates:	Test item: 5 per test trial Reference item: 1 per test trial
Number of replicates per test group:	40 (one replicate = one larva)
Number of larvae/per replicate:	1

In two trials the effects on the pre-imaginal mortality and the reproduction of residues of BYI 02960 SL 200 on the ladybird beetle *Coccinella septempunctata* were determined. The test item was applied onto detached bean leaves (*Phaseolus vulgaris*).

In the first trial, test item rates of 8, 17, 35, 72 and 150 g a.i./ha, respectively, were assessed in comparison to a de-ionized water control treatment. A toxic reference (active substance: dimethoate) applied at 12 g a.i./ha was included to indicate the relative susceptibility of the test organisms and the test system.

As no effects occurred in all test item rates of the first trial a second trial with dose rates of 100, 160, 250, 380 and 600 g a.i./ha, respectively, was conducted. A re-analyzed batch of BYI 02960 SL 200 (g/L) was tested, specified by sample description: TOX 08854-00; specification No.: 102000021884-01; batch ID: 2009-001253 (analysed content of active ingredient BYI 02960: 17.0% w/w); density: 1.174 g/mL.

Furthermore a new batch of dimethoate was used as toxic reference for this trial and was applied at 12 g a.i./ha.

The suspensions for the test and reference item were applied to detached *Phaseolus vulgaris* leaves. After application one larva was added to each test unit (within the first hour after application).

3. Observation and measurements

In both trials the preimaginal mortality of 40 larvae was assessed until the hatch of the imagines (up to 13 days in the first and 17 days in the second trial). All exposure units were assessed daily and the condition of the ladybird larvae was recorded. The larvae were fed daily with fresh aphids (*A. pisum*) ad libitum. At every feeding session dead aphids and exuviae from earlier feeding sessions were removed, in order to maintain a constant contact between the larvae and the treated surface.

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The fertility and fecundity of the surviving hatched adults were then evaluated over the period of 17 days in the both study trials. Once the larvae had pupated and the pupae hatched, the emerged beetles were transferred to glass jars and the sex of the beetles was determined. Fresh aphids were fed when required. Sheets of black paper were offered to the beetles for egg-laying. The sheets were checked daily and freshly laid eggs were cut out of the paper and stored in petri dishes.

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 LR_{50} value was calculated using Probit analysis with the data of the second trial.

RESULTS AND DISCUSSION

A. Environmental Conditions

Beetles were kept under conditions which are summarized as follows:

Test temperature:	23.0-26.0°C in the first trial (short increase <2h to 27.5°C 23.5-26.0°C in the second trial
Relative humidity:	65 - 80 % in the first trial (short decrease <2h to 46%) 67 - 89% in the second trial (short decrease <2h to 59%)
Photoperiod:	16 hours light / 8 hours dark
Light intensity	1380-5391 Lux in the first trial 1305 – 6017 Lux in the second trial

B. Biological Findings

A summary of effects of BYI 02960 SL 200 on mortality and reproduction of ladybird beetle *Coccinella septempunctata* exposed on detached bean leaves is given below:

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Table 10.5.2- 04: Effects of BYI 02960 SL 200 (g/L) on mortality and reproduction of *Coccinella septempunctata*

Test item		BYI 02960 SL 200 (g/L)				
Test organism		*Coccinella septempunctata*				
Exposure on:		Detached bean leaves				
Trial 1						
		Mortality [%]			Reproduction	
Treatment	**g a.i./ha**	**Uncorr.**	**Corr.**	**P-Value(*)**	**Fertile eggs per female and day**	**Fertility [%hatching rate]**
Control	0	22.5			10.4	92.0
Test item	8	32.5	12.9	0.906 n.sign.	15.5	91.4
Test item	17	25.6	4.1	1.000 n.sign.	17.6	95.7
Test item	35	15.0	-9.7	1.000 n.sign.	9.5	87.3
Test item	72	25.0	3.2	1.000 n.sign.	15.5	93.5
Test item	150	47.5	32.3	0.085 n.sign.	21.6	95.4
Reference item	12	92.5	90.3		n.a.	n.a.
* Fisher`s Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm n.a. not assessed n.sign. not significant						
Trial 2						
		Mortality [%]			Reproduction	
Treatment	**g a.i./ha**	**Uncorr.**	**Corr.**	**P-Value(*)**	**Fertile eggs per female and day**	**Fertility [%hatching rate]**
Control	0	22.5			13.9	91.9
Test item	100	30.0	9.7	0.306 n.sign.	16.1	92.0
Test item	160	37.5	19.4	0.222 n.sign.	21.0	92.3
Test item	250	57.5	45.2	0.004 sign.	25.6	91.3
Test item	380	77.5	71.0	<0.001 sign.	n.a.	n.a.
Test item	600	97.5	96.8	<0.001 sign.	n.a.	n.a.
Reference item	12	100.0	100.0		n.a.	n.a.
LR_{50}: 273.9 g a.i./ha; 95 % Confidence Interval: (185.9 - 330.8) (calculated with Probit analysis) * Fisher`s Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm n.a. not assessed n.sign. not significant sign. Significant						

1. Pre-imaginal mortality

In the first trial no statistically significant corrected mortality could be observed in all tested rates of BYI 02960 SL 200. For the reference item a corrected pre-imaginal mortality of 90.3% occurred.

In the second trial no statistically significant corrected pre-imaginal mortality could be observed in the two lowest rates of BYI 02960 SL 200 tested. In all other tested rates a statistically significant corrected mortality could be observed. In the reference item a corrected mortality of 100% occurred.

2. Reproduction

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In the first trial the mean number of fertile eggs per female and day for the control during the test period was 10.4. The mean number of fertile eggs per female and day for the 8, 17 and 35 g a.i./ha rate was 15.5, 17.6 and 9.5, respectively. A mean number of 15.5 and 21.6 fertile eggs per female and day, respectively, could be found in the 72 and 150 g a.i./ha rate.

In the second trial the mean number of fertile eggs per female and day for the control during the test period was 13.9. The mean number of fertile eggs per female and day for the 100 and 160 g a.i./ha rate was 16.1 and 21.0, respectively. A mean number of 25.6 fertile eggs per female and day could be observed in the 250 g a.i./ha rate of BYI 02960 SL 200.

C. Validity Criteria

The validity criteria are based on those of the laboratory method with glass plates ([redacted] ET AL., 2000). In both trials of the study the mortality in the control group was ≤ 30% and the toxic reference resulted in ≥ 40% corrected mortality. The average number of fertile eggs per female and day in the control was ≥ 2. Therefore the results of this study can be considered as valid.

D. Biological Endpoints Derived

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

LR_{50}: 273.9 g a.i./ha; 95 % Confidence Interval: 185.9 – 330.8 g a.i./ha

CONCLUSION

The effects of BYI 02960 SL 200 residues on larvae of the ladybird beetle *Coccinella septempunctata* can be quantified as an LR_{50} of 273.9 g a.i./ha (95% CI: 185.9 to 330.8 g a.i./ha). Because the reproductive performance was within the historical data base for control beetles (≥ 2 fertile eggs per female and day, [redacted] ET AL. 2000) this parameter is considered as not affected at all these test item rates for which reproduction was assessed.

Report:	**KIIIA1 10.5.2/04; [redacted]. (2010)**
Title:	Chronic toxicity (ER50) of BYI 02960 SL 200 (g/L) to the rove beetle *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) under extended laboratory conditions
Report No:	CW09/072
Document No:	M-384433-01-2
Guidelines:	**[redacted] ET AL. (2000); [redacted] ET AL. (2001)**
Deviations from guideline:	**At the beginning of the reproduction phase the humidity decreased to 52 - 56% for a period of approx. five days. This had no negative impact on the study outcome as all validity criteria where met.**
GLP:	**Yes (certified laboratory)**

Executive Summary

The purpose of this study was to determine possible impacts of the test item on the reproductive capacity of the rove beetle *Aleochara bilineata* in an extended laboratory test after exposure to BYI 02960 SL 200 (Sample description: FAR01438-00 (Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 102000021884)) applied at different application rates onto sandy soil (LUFA 2.1).

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In a first trial, test item rates of 10, 21, 45, 95, and 200 g a.i./ha were assessed in comparison to a deionised water control treatment. A toxic reference (active substance: dimethoate) applied at 788.8 g a.i./ha was included to indicate the relative susceptibility of the test organisms and the test system. As no effects occurred in all test item rates of the first study trial, a second limit trial with 300 g a.i./ha was conducted. A new batch of dimethoate was used as toxic reference for this trial and was applied at 788.8 g a.i./ha.

In both trials, 80 adults of *Aleochara bilineata* (4 replicates of 10 females and 10 males per test group) were exposed to the spray residues of the test item, reference item and control, respectively, for a period of four weeks. During the assessments the rove beetles were fed with deep frozen larvae of *Tenebrio molitor*. At day 7, 14 and 21 after application approximately 500 onion fly pupae (*Delia antiqua*) were added and carefully mixed with the substrate of each exposure unit so that they could be parasitized by beetle larvae. The number of hatched beetles of the F1 generation was recorded over a period of 46 days in the first trial and 39 days in the second study trial.

No reduction of reproductive capacity occurred at all rates tested. The ER_{50} was estimated to be >300 g a.i./ha.

Material and Methods

A. Materials

1. Test material

Test item:	BYI 02960 SL 200
Specification No.:	102000021884
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Material number:	79718845
Sample description:	FAR01438-00
Batch No.:	2009-001253
Nominal content of active substance:	BYI 02960: 200 g/L
Analytical content of active substance:	BYI 02960: 17.0% w/w, 199.8 g/L, according to certificate of analysis
Density:	1.175 g/mL
Stability of test compound:	Approved until 03/2010 (storage at +2 °C to +30 °C)

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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
Negative control:	deionized water
Positive control:	Reference substance: Dimethoate EC 400 g/L (428.5 g/L)

3. Test organism

Species:	*Aleochara bilineata*
Common name:	Rove beetle
Age:	4 days
Source of test organism:	The onion fly pupae, *Delia antiqua* MEIGEN, used as hosts for *Aleochara bilineata* were supplied by [redacted]. The original source of the beetles had been the [redacted] and the [redacted], Denmark. The rearing in the laboratory of De Groene Vlieg started 1995 (host: pupae of *Delia antiqua*; rearing conditions: 20 - 21 °C, natural day length, food: dead larvae of *Delia antiqua*). The larvae of *Tenebrio molitor* L. (yellow mealworm), which were used as food for the beetles were obtained from [redacted]

B. Study design and methods

1. In life dates — October 14, 2009 to March 31, 2010

2. Design of the test

Trial 1:

Number of test groups:	7 (control, test item and reference item)
Number of application rates:	Test item: 5 Reference item: 1
Number of replicates per test group:	4
Number of beetles per replicate:	10 male and 10 female adult beetles

Trial 2:

Number of test groups:	3 (control, test item and reference item)
Number of application rates:	Test item: 1 Reference item: 1
Number of replicates per test group:	4
Number of beetles per replicate:	10 male and 10 female adult beetles

In two trials, the effects of BYI 02960 SL 200 on the reproductive capacity of the rove beetle *Aleochara bilineata* were determined. The test item was applied onto sandy soil (LUFA 2.1).

Test item rates of 10, 21, 45, 95, and 200 g a.i./ha, respectively (first trial) and 300 g a.i./ha (second trial) were assessed in comparison to a deionised water control treatment.

A toxic reference (active substance: dimethoate) applied at 788.8 g a.i./ha was included to indicate the relative susceptibility of the test organisms and the test system.

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On the application day the sex of the beetles was determined by observing the mating behaviour. Directly after treatment ten pairs of male and female adult beetles were added impartially to each exposure unit by placing them on the treated substrate. The units were closed with gauze lids and transferred to a controlled environmental room.

Approximately one hour after application, the beetles were fed with larvae of *Tenebrio molitor* and then in 2 to 3 day intervals up to day 28 after application. At day 7, 14 and 21 after application approximately 500 onion fly pupae (*Delia antiqua*) were added. The number of pupae was determined by weight on each occasion.

At day 28 the beetles were removed from the exposure units and discarded. The soil containing the parasitized onion fly pupae was allowed to dry for seven days by removing the lids from the exposure units.

At day 35 after application the pupae were removed from the substrate by a sieve and by flushing with water. After drying the pupae were placed in hatching cages (each replicate separately) and incubated in a controlled environmental room.

3. Observation and measurements

The number of hatched beetles of the F1 generation was recorded over a period of 46 days in the first trial and 39 days in the second study trial. The number of hatched beetles was recorded daily. The test was terminated when the hatching rate of *Aleochara bilineata* beetles in the control group had fallen below two beetles per replicate per day.

From these data the endpoint reproductive capacity was calculated.

4. Statistics

The computer program SAS (Version 9.1.3, 2002-2003) was used to perform the statistical analyses.

The reproduction data were tested for normal distribution using the Shapiro-Wilk test and for homogeneity using the Levene test.

As the reproduction data in both trials were normally distributed and homogenous one-way ANOVA and the Williams test (one-sided; $\alpha = 0.05$) were used.

RESULTS AND DISCUSSION

A. Environmental Conditions

Beetles were kept under conditions which are summarized as follows:

Test temperature:	19.0-21.0°C in the first trial 19.0-20.0°C in the second trial
Relative humidity:	60 - 69% in the first trial (short decreases <2h to 59%) 60 - 87% in the second trial (short decrease <2h to 43%) At the beginning of the reproduction phase the humidity decreased to 52 - 56% for a period of approx. five days due to technical problems. This had no negative impact on the study outcome as all validity criteria where met
Photoperiod:	16 hours light / 8 hours dark
Light intensity	347-683 Lux in the first trial 360 – 622 Lux in the second trial

B. Biological Findings

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A summary of the effects of BYI 02960 SL 200 applied onto soil on the reproductive capacity of the rove beetle *Aleochara bilineata* is given in the table below:

Table 10.5.2- 05: Effects of BYI 02960 SL 200 (g/L) on the reproductive capacity of the rove beetle *Aleochara bilineata*

Test item		BYI 02960 SL 200 (g/L)		
Test organism		*Aleochara bilineata*		
Exposure on:		Soil		
Trial 1				
		Reproductive capacity		
Treatment	g a.i./ha	Hatched beetles per introduced female (number)	Red. rel. to control [%]	P-Value(#)
Control	0	76.4		
Test item	10	70.6	7.6	0.219 n.sign.
Test item	21	62.5	18.2	0.261 n.sign.
Test item	45	76.7	-0.4	0.279 n.sign.
Test item	95	66.3	13.2	0.289 n.sign.
Test item	200	63.7	16.5	0.296 n.sign.
Reference item	788.8	27.1	64.5	
# one-way ANOVA, Williams test (one-sided) n.sign. not significant				
Trial 2				
		Reproductive capacity		
Treatment	g a.i./ha	Hatched beetles per introduced female (number)	Red. rel. to control [%]	P-Value(#)
Control	0	71.2		
Test item	300	62.0	13.0	0.322 n.sign.
Reference item	788.8	21.5	69.8	
ER_{50} > 300 g a.i./ha # one-way ANOVA, Williams test (one-sided) n.sign. not significant				

The mean number of hatched beetles per replicate in the control was 764 in the first and 712 in the second study trial. The mean number of hatched beetles per replicate in the reference item group was reduced to 64.5% in the first and to 69.8% in the second study trial, compared to the respective control group.

C. Validity Criteria

The validity criteria was based on those of the laboratory and extended laboratory method ([redacted] ET AL., 2000). In the control group of each study trial the average number of hatched beetles of the F1-generation per replicate was > 400. The reduction of reproductive capacity of the reference item group of each study trial relative to control was ≥ 50%. Therefore the results of both study trials can be considered as valid.

D. Biological Endpoints Derived

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

ER_{50}: >300 g a.i./ha

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CONCLUSION

The effects of BYI 02960 SL 200 on the reproductive capacity of the rove beetle *Aleochara bilineata* can be quantified as an ER_{50} of >300g a.i./ha. No reduction of reproductive capacity occurred at all rates tested.

IIIA 10.5.3 Effects on non-target terrestrial arthropods in semi-field tests

Report:	**KIIIA 10.5.3/01; [redacted]. (2010)**
Title:	Toxicity to the parasitoid *wasp Aphidius rhopalosiphi* (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) in an extended laboratory test (under semi-field conditions aged residues on *Zea mays*); BYI 02960 SL 200 (g/L)
Report No:	CW10/018
Document No:	M-396372-01-2
Guidelines:	**[redacted] ET AL. (2000), [redacted] ET AL. (2009) modified: Use of treated maize plants, wasps exposed to freshly applied and under semi-field conditions aged residues on cut leaves. [redacted] ET AL. (2001)**
Deviations:	**None**
GLP:	**Yes (certified laboratory)**

Executive Summary

The objective of this study was to investigate the lethal and sub lethal toxicity of BYI 02960 SL 200 (Sample description: TOX08854-00 (Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 102000021884-01)) to the parasitoid wasp *Aphidius rhopalosiphi* when exposed to aged residues of the test item on maize.

The test item was applied on potted maize plants two times with 250 g a.i./ha in 400 L water/ha with a time interval of 10 days. The control was treated with deionised water in the same way as the test item. A toxic reference (active substance: dimethoate) was applied at 3 g a.i./ha in 400 L water/ha on the second application day of the test item on potted maize plants as well. For the further exposure dates it was applied directly on the cut maize leaves.

Aging of the spray residues of the test item on the potted maize plants took place under semi-field conditions with rain protection during the whole study. Parasitoid wasps (*Aphidius rhopalosiphi*) were exposed to residues on treated leaf surfaces aged for 0, 14, 28, 42, 49 and 56 days (6 bioassays). Repellency of the test item was assessed during the initial 3 hours after the release of the females. Mortality of 30 females (6 replicates with 5 wasps per test group) was assessed 2, 24 and 48 h after exposure in all bioassays. The reproductive performance was assessed in the bioassays started on day 42, 49 and day 56 after the second application of the test item. For this 15 impartially chosen females from the water control and the test item group were each transferred to a cylinder containing untreated barley seedlings infested with Rhopalosiphum padi for a period of 24 h. The number of mummies was assessed 11 days later.

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A corrected mortality of 100% of the test item was found in the first bioassay started on the day of the second application. A second bioassay was started 14 days after the last application and showed 90% corrected mortality in the test item group after 48 h of exposure. In a third bioassay (day 28), a corrected mortality of 76.7% occurred. A fourth bioassay was started on day 42 which resulted in a corrected mortality of 29.6%. In this bioassay reproduction was assessed, which showed 89.9% reduction in reproductive success relative to the control. In a fifth bioassay started on day 49 after the last application 20% corrected mortality was observed and the reduction in reproduction was 37.6%. A final bioassay on day 56 confirmed the results of the fifth bioassay. The corrected mortality was 6.7% and no reduction of reproductive success (0.6%) was found.

Statistically significant repellent effects of the test item were observed in the first, second and third bioassay. No significant repellent effects were observed in the fourth, fifth and sixth bioassay.

MATERIAL AND METHODS

A. Materials

1. Test material:

Test item:	BYI 02960 SL 200
Specification No.:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Material number:	79718845
Sample description:	TOX 08854-00
Batch No.	2009-001253
Nominal content of active substance:	BYI 02960: 200 g/L
Analytical content of active substance:	BYI 02960: 17.0% w/w, 199.8 g/L according to certificate of analysis
Density:	1.174 g/mL
Stability of test compound	Approved until 02.12.2010 (storage at +2 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 (414.8 g/L)

3. Test organism

Species	Parasitoid wasp *Aphidius rhopalosiphi*
Age	48 h
Source of test organism	*Aphidius rhopalosiphi* used for testing were supplied [redacted]
Source of host organism	Cereal and maize plants were provided by the horticultural group of BCS Global Biology Herbicides. *Rhopalosiphum padi* (aphids used for parasitation in the reproduction assessment) were taken from the breeding of the testing facility.

B. Study design and methods

1. In life dates May 7 to July 26, 2010

2. Design of the test

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Number of test groups:	3 (control, test and reference item)
Number of application rates:	Test item: 1 Reference item: 1
Number of replicates per test group:	6 (one replicate = one exposure unit)
Number of larvae/per replicate:	5

A soluble concentrate formulation of BYI 02960 SL 200 (g/L) was tested, specified by sample description: FAR 01438-00; specification no.: 102000021884; batch ID: 2009-001253 [analysed content of active ingredient BYI 02960: 17.0 %w/w, 199.8 g a.i /L]; density: 1.175 g/mL.

The test item was applied two times with 250 g a.i./ha in 400 L water/ha on potted maize plants. The time interval between the first and second application was 10 days.

Aging of the spray residues of the test item on the potted maize plants took place under semi-field conditions with rain protection during the whole study. The control was treated with deionised water in the same way as the test item. A toxic reference (active substance: dimethoate) was applied at 3 g a.i./ha in 400 L water/ha on the second application day of the test item on potted maize plants as well. For the further exposure dates it was applied directly on the cut maize leaves (with 3 g a.i./ha in 400 L water/ha). It was included to indicate the relative susceptibility of the test organisms and the test system.

After the last application or at the relevant interval afterwards pieces of maize leaves (approx. 25 cm long) were cut and transplanted in a pot The pot was filled with quartz sand so that the leaf stood in an upright position and enclosed within a clear polyacrylic cylinder. For the mortality assessment five female test organisms were introduced per cylinder. For the reproduction assessment in the fourth, fifth and sixth bioassay 15 females per test group were impartially selected from the surviving females and were kept individually in a cylinder with barley seedlings infested with aphids (*R. padi*). After one day the wasps were removed and the seedlings kept for 11 more days. The parasitation rate was determined by counting the number of developed mummies for each individual female wasp.

3. Observation and measurements

Mortality of 30 females (6 replicates with 5 wasps per test group) was assessed 2, 24 and 48 h after exposure in all bioassays. The condition of the animals was observed as:

- live (alive and apparently unaffected)
- affected (showing reduced co-ordination or any abnormal behaviour)
- moribund (unable to walk, but still moving legs or antennae)
- dead (no longer moving

Repellency of the test item was assessed during the initial 3 hours after the release of the females. Five separate observations were made at 30-minute intervals starting 15 - 30 minutes after the introduction of all wasps. Each wasp was recorded as being on the:

Plants: on the treated leaf

Cylinder: on the walls of the cylinder

Soil: on the sand below the leaf

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Reproductive performance was calculated for each replicate and expressed as mummies per female. Only results for wasps found alive after the 24-h parasitation period were used for the reproduction analysis.

4. Statistics

The computer program SAS (Version 9.1.3, 2002-2003) was used to perform the statistical analyses.

The mortality data of all bioassays were analysed for significance using the Fisher Exact test (one-sided with Bonferroni-Holm adjustment; α = 0.05), which is a distribution-free test method and does not require testing for normality or homogeneity prior analysis.

The repellency data were tested for normal distribution using the Shapiro-Wilk test and for homogeneity using the Levene test.

In the first, third and fourth bioassay the repellency data were normally distributed and homogenous therefore one-way ANOVA and the Williams test (one-sided; α = 0.05) were used. In the second bioassay one-way ANOVA and the Dunnett test (one-sided, α = 0.05) were used, with the repellency data being normally distributed and homogenous. In the fifth bioassay one-way ANOVA and the Dunnett test (two-sided, α = 0.05) were used, with the repellency data being normally distributed and homogenous. As the repellency data in the sixth bioassay were not normally distributed the Wilcoxon test (one-sided with Bonferroni-Holm adjustment; α = 0.05) was used.

RESULTS AND DISCUSSION

A. Environmental Conditions

Test temperature:	19.5-22.0°C
Relative humidity:	61 – 90 %
Photoperiod:	16 hours light / 8 hours dark
Light intensity	415 - 1165 Lux during mortality phase
	1058 - 4030 Lux during parasitation phase
	2100 – 13840 Lux during reproduction phase

B. Biological Findings

The bioassays were started on the day of the second application of the test item and 14, 28, 42, 49 and 56 days after the second application, respectively. These bioassays resulted for the test item group in 100.0, 90.0, 76.7, 29.6, 20.0 and 6.7% corrected mortality, respectively.

During the observations in the initial three hours of each bioassay statistically significant repellent effects could only be observed in the first, second and third bioassay; probably due to the high effects of the test item in these bioassays. In all other bioassays the number of wasps in the test item group found sitting on the leaf was not statistically different compared to those in the control group.

Due to the observed corrected mortality (≥ 50%) in the first, second and third bioassay the effects on reproduction were only assessed in the fourth, fifth and sixth bioassay (started at 42, 49 and 56 DAA).

A statistically significant reduction in reproductive success relative to the control of 89.9% was found in the fourth bioassay (42 DAA). In the fifth bioassay (49 DAA) a reduction in reproduction of 37.6% was observed which was not statistically different. No effect on reproduction (0.6%) was observed in the sixth bioassay (56 DAA).

Detailed results are presented below:

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Table 10.5.3- 1: Effects of BYI 02960 SL 200 (g/L) on mortality and reproduction of *Aphidius rhopalosiphii*

Test item:	**BYI 02960 SL 200 (g/L)**					
Application rate:	2 x 250 g a.i./ha					
Test organism:	*Aphidius rhopalosiphi*					
Exposure on:	Dried spray deposits on maize leaves (from treated maize plants)					
Start bioassay:	**0 DAA[a]**	**14 DAA[a]**	**28 DAA[a]**	**42 DAA[a]**	**49 DAA[a]**	**56 DAA[a]**
	Mortality (%) after 48 h					
Control:	6.7	0.0	0.0	10.0	0.0	0.0
Test item:	100.0	90.0	76.7	36.7	20.0	6.7
Reference item:	100.0	100.0	100.0	100.0	100.0	100.0
	Corrected Mortality (%)					
Test item:	**100.0** (p < 0.001, significant[b])	**90.0** (p < 0.001, significant[b])	**76.7** (p < 0.001, significant[b])	**29.6** (p = 0.015, significant[b])	**20.0** (p = 0.012 significant[b])	**6.7** (p = 0.246, not sign.[b])
Reference item:	100.0	100.0	100.0	100.0	100.0	100.0
	Repellency (mean values) **% Wasps on plant**					
Control:	58.2	48.2	62.0	50.0	46.0	44.5
Test item:	18.6	25.3	43.0	31.3	54.7	47.2
Reference item:	56.7	37.0	55.5	45.0	40.8	45.8
	Rel. to control (%)					
Test item	**68.1** (p = 0.007, significant[c])	**47.5** (p = 0.006, significant[d])	**30.6** (p = 0.012, significant[c])	**37.3** (p = 0.062, not sign.[c])	**-18.8** (p = 0.051, not sign.[f])	**-6.0** (p = 0.416, not sign.[g])
Reference item:	2.6	23.2	10.5	10.0	11.2	-3.0
	Reproduction (Number of mummies per female)					
Control:	n.a.	n.a.	n.a.	**15.4**	**18.0**	**24.1**
Test item:	n.a.	n.a.	n.a.	**1.6**	**11.2**	**24.0**
	Reduction rel. to control (%)					
Test item:	n.a.	n.a.	n.a.	**89.9** (p < 0.001, significant[e])	**37.6** (p = 0.181, not sign.[c])	**0.6** (p = 0.499, not sign.[c])

n.a.: not assessed; a Days after 2nd application; b Fisher´s Exact test (one-sided); c one-way ANOVA, Williams test (one-sided); d one-way ANOVA, Dunnett test (one-sided); e Welch test (one-sided); f Dunnett test (two-sided); g Wilcoxon test (one-sided)

C. Validity Criteria

In all bioassays the control mortality was ≤ 10% and the corrected mortality of the toxic reference group was ≥ 50%. The mean reproduction per female in the control of the fourth, fifth and sixth bioassay was ≥ 5 mummies per female with one wasp producing no mummies in the fourth bioassay and zero wasp producing no mummies in the fifth and sixth bioassay.

Therefore the results of this study can be considered as valid (validity criteria based on the guideline for an extended laboratory test ([redacted] ET AL., 2009)).

CONCLUSION

After 2 applications of 250 g a.i./ha of BYI 02960 SL 200, the effects on survival of *Aphidius rhopalosiphi* dropped below 50% after an aging period of 42 days. No effects > 50% were observed for either mortality or reproduction after an aging period of 49 days.

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Report:	**KIIIA1 10.5.3/02; [redacted] (2010)**
Title:	Effects of BYI 02960 SL 200 (g/L) on the Predatory Bug *Orius laevigatus*, Extended Laboratory Study - Aged Residue Test
Report No:	EBRVP101
Document No:	M-394033-01-2
Guidelines:	**[redacted] et al., 2000 (modified for exposure on natural substrate)**
Deviations from guideline:	**None**
GLP:	**Yes (certified laboratory)**

Executive Summary

The purpose of this study was to determine the toxicity of BYI 02960 SL 200 (Sample description: TOX08854-00 (Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 102000021884-01)) on nymphs of the predatory bug *Orius laevigatus* in the laboratory by contacting fresh and aged spray residues on apple leaves (*Malus domestica*) compared to a water treated control and to a reference item. Additionally, an assessment for significant sublethal effects on the reproductive performance (fecundity and fertility) of the survivors was made.

The test item was applied two times at 250 g a.i./ha in an interval of 10 days. The study encompassed 3 treatment groups (1 dose rate of the test item, control, reference item dimethoate). A 1st bioassay was started on the day of the 2nd application, the 2nd bioassay was started 14 days, the 3rd bioassay 28 days and the 4th bioassay 42 days after the 2nd application, respectively, with 50 replicates, each containing 1 nymph. The nymphs were exposed to dried residues on treated leaf surfaces (apple leaves). Mortality checks were carried out regularly up to 15 days after begin of the bioassay. In addition, in the 3rd and 4th bioassay the reproduction performance, i.e. egg deposition (fecundity) and nymphal hatching rate (fertility), was determined for the control and the test item treatment group (2 checks, each lasting 2 days, nymphal hatching rate determined from the 1st check).

On the day of the last application (1st bioassay DAT 0) and on day 14 after the last application (2nd bioassay DAT 14) *O. laevigatus* showed a corrected mortality of 100% and 75.6%, respectively, when exposed to residues of the test item. The 3rd bioassay 28 days after the last application showed a corrected mortality of 24.5%. In this bioassay the fecundity and fertility of *O. laevigatus* were not affected. In the 4th bioassay conducted 42 days after the last application, mortality, fecundity and fertility of *O. laevigatus* were not affected when exposed to aged residues of BYI 02960 SL 200.

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MATERIAL AND METHODS

A. Material

1. Test material

Test item:	BYI 02960 SL 200
Specification No.:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Material number:	79718845
Sample description:	TOX 08854-00
Batch No.:	2010-001067
Nominal content of active substance:	BYI 02960: 200 g/L
Analytical content of active substance:	BYI 02960: 17.0% w/w, 201.0 g/L according to certificate of analysis
Density:	1.175 g/mL
Stability of test compound:	Approved until 06/2012 (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
Negative control:	Deionized water
Positive control:	Dimethoate EC 400 (analysed content: 414.8 g a.i./L)

3. Test organism

Species:	*Orius laevigatus*
Common name:	Predatory bug
Age:	4 - 5 day old nymphs (2nd instar)
Source of test organism:	*Orius laevigatus* used for testing were supplied by [redacted]
Source of host organism:	Exposure units: Detached leaves (leaves were cut from field treated apple plants [*Malus domestica* 'M 9 Unterlage'], grown under field conditions, non-GLP, without any treatment before application of the test item. Oviposition units: Detached primary leaves of untreated bean plants (*Phaseolus vulgaris*)

B. Study design and methods

1. In life dates: May 28 to August 12, 2010

2. Design of the test

Number of test groups:	3 (control, test and reference item)
Number of application rates:	Test item: 2 Reference item: 2
Number of replicates per test group:	50 (one replicate = 1 nymph)
Number of nymphs per replicate:	1

The test item was sprayed onto apple plants grown in the field via field spraying equipment and air dried afterwards outdoors under natural conditions. The control and the reference item were sprayed one time in parallel with the 2 nd application of the test item onto the apple plants via field spraying equipment and air dried afterwards outdoors under natural conditions, too. In addition the reference item was applied on apple leaves collected in the field on each start day of a bioassay via laboratory spraying equipment.

In the exposure phase of the test, 50 2nd instar nymphs per treatment group were placed in exposure units and exposed to treated plants (leaf discs) for 11 – 15 days. The 1st bioassay was carried out with freshly dried residues on the day of the 2nd application. The 2nd, 3rd and 4th bioassay on aged residues were started on day 14, 28 and 42 after the 2nd application, respectively.

In the 3rd and 4th bioassay fecundity assessment (oviposition) started 4 days after the 80% criterion (when 80% of the animals in the control became adult) was met or on day 14. Females were transferred to reproduction units. Two fecundity assessments over a time period of 2 days each were done. After transferring the female bugs to the oviposition substrate, they were left undisturbed for 2 days. After these 2 days the number of eggs laid was counted (1st check). After the assessment the female bugs were transferred to new oviposition substrate and were left undisturbed for another 2 days. The number of eggs laid within these 2 days was counted too (2nd check). The first batch of eggs (1st check) was retained to determine the hatching rate of the eggs (fertility) up to 6 days after 1st check was finished.

3. Observation and measurements

For the mortality assessment, numbers of living, dead and escaped predatory bugs were assessed at least 2 times a week. The mortality assessment was finished on day 15 latest.

For the fecundity assessment, the number of eggs produced per female was recorded for each fecundity check. Each test unit was examined for the number of eggs laid on the egg laying substrate and for dead, surviving and missing females.

To assess fertility, substrates with eggs laid during the 1st fecundity check were kept up to 6 days to determine the number of hatched eggs.

4. Statistics

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ToxRat® Solutions GmbH.

Mortality data were analysed for significance using the Fisher's Exact Test, which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis.

Fecundity and fertility was tested for normal distribution and homogeneity of variance using the Kolmogorov-Smirnov test ($\alpha = 0.05$) and the Bartlett's test ($\alpha = 0.05$).

Because for the 3rd bioassay the fecundity data were normally distributed and homogeneous, the Student t-test for homogeneous variances (pair wise comparison, one-sided, $\alpha = 0.05$) was used. As the data for the fertility assessment were not normally distributed and homogeneous the Mann-Whitney U test (pair wise comparison, one-sided, $\alpha = 0.05$) was used.

Because fertility and fecundity data were normally distributed and homogeneous, the Student t-test for homogeneous variances (pair wise comparison, one-sided, $\alpha = 0.05$) was used for the 4th bioassay.

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RESULTS AND DISCUSSION

A. Environmental Conditions

Test temperature:	23.0-27.0°C
Relative humidity:	61 - 90%
Photoperiod:	16 hours light / 8 hours dark
Light intensity	340-1130 Lux

B. Biological Findings

After an application of two times 250 g a.i./ha of BYI 02960 SL 200 with an application interval of 10 days *O. laevigatus* was affected showing a corrected mortality 100% and 75.6% when exposed to residues of the test item starting on the day of the last application (1st bioassay DAT 0) and on day 14 after the last application (2nd bioassay DAT 14), respectively.

In the 3rd bioassay, 28 days after the last application, the corrected mortality was 24.5%. In this bioassay the fecundity and fertility were not affected.

In the 4th bioassay conducted 42 days after the last application mortality, fecundity and fertility were not affected.

After 2 applications, effects on survival of *O. laevigatus* dropped below 50% after an ageing period of 28 days. No effects on reproduction were observed after this aging period.

Detailed results are presented below:

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Table 10.5.3- 2: Effects on *Orius laevigatus* exposed to BYI 02960 SL 200 in an extended laboratory trial

	Rate 1) [g a.i. /ha]	Mortality 2) [%]	Mortality corr. 3) [%]	Fecundity 4) [eggs/female /day]	Effect on Fecundity [%]	Fertility 7) [% nymphal hatching rate]	Effect on Fertility 5) [%]
1st bioassay: test start on the day of 2nd application							
Control	--	14.0	--	--	--	--	--
BYI 02960 SL 200	2 x 250	100.0 *	100.0	--	--	--	--
Perfekthion	90.0 (mL/ha)	100.0 *	100.0	--	--	--	--
2nd bioassay: test start 14 days after the 2nd application							
Control	--	10.0	--	--	--	--	--
BYI 02960 SL 200	2 x 250	78.0 *	75.6	--	--	--	--
Perfekthion 4)	25.0 (mL/ha)	100.0 *	100.0	--	--	--	--
3rd bioassay: test start 28 days after the 2nd application							
Control	--	2.0	--	7.2	-	90.8	-
BYI 02960 SL 200	2 x 250	26.0 *	24.5	5.5 n.s.	23.0	96.7 n.s.	-6.5
Perfekthion 6)	25.0 (mL/ha)	100.0 *	100.0	--	--	--	--
4th bioassay: test start 42 days after the 2nd application							
Control	--	18.0	--	4.8	-	88.3	--
BYI 02960 SL 200	2 x 250	26.0 n.s.	9.8	3.1 n.s.	34.7	76.9 n.s.	12.9
Perfekthion 6)	25.0 (mL/ha)	100.0 *	100.0	--	--	--	--

1) Application rate in 400 L water/ha; application was done in the field under outdoor conditions, the test item was applied two times in an interval of 10 days
2) Pre-imaginal mortality after exposure to treated leaf surfaces (Fisher's Exact Test; α = 0.05: * = significant)
3) Corrected pre-imaginal mortality according to Abbott and improvements by Schneider-Orelli
4) Mean number of eggs per female per day from 2 checks, each lasting 2 days (Student t-test; α = 0.05: n.s. = not significant)
5) Mean nymphal hatching rate (Mann-Whitney U-test; α = 0.05: n.s. = not significant)
6) Application rate in 200 L water/ha; application was done in the laboratory
7) Mean nymphal hatching rate (Student t-test; α = 0.05; n.s. = not significant)

C. Validity Criteria

In all bioassays the control mortality was ≤ 25% and the corrected mortality of the toxic reference group was 100%. The validity criteria for fecundity (more than 2 eggs per female per day (mean value) and no more than 5 bugs producing zero values in the control) and for fertility (more than 70% of the eggs hatching successfully in the control) were met.

Therefore the results of this study can be considered as valid.

CONCLUSION

After 2 applications of 250 g a.i./ha of BYI 02960 SL 200, the effects on survival of *O. laevigatus* dropped below 50% after an aging period of 28 days. No effects on reproduction were observed after this aging period.

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IIIA1 10.5.4 Field tests on arthropods species

Report:	**KIIIA1 10.5.4/01; [redacted] S., [redacted] F.; 2012a**
Title:	A field study to assess the effects of BYI 02960 SL 200 g/L on the non-target, surface- and plant-dwelling, arthropod fauna of a grassland habitat (off-crop) in The Netherlands during summer
Report No:	B154FFN
Document No:	M-425092-01-2
Guidelines:	**IOBC ([redacted], 1992), ANONYMOUS (1992), [redacted] (1998), IOBC, BART AND EPPO JOINT INITIATIVE ([redacted] ET AL., 2000, 2001), [redacted] ET AL., 2010**
Deviations from guideline:	**Use of true off-crop habitat (grassland habitat with little agricultural input) representing a realistic worst-case scenario** **Use of NOER-type study (No Observed Effect Rate), making the results applicable to any product use pattern.**
GLP:	**Yes (certified laboratory)**

Executive Summary

BYI 02960 SL 200 is an insecticide with a wide range of uses. This field study was designed to assess the potential adverse effects on Non-Target Arthropods (NTA) in off-crop habitats that might occur at various distances from a treated area for current and future use pattern of the test item. The study was set up to enable an assessment of community- and population level ecotoxicological standards, in particular the NOER (No Observed Effect Rate), the NOEAER and the LOEAER (No and Lowest Observed Ecologically Adverse Effect Rate, respectively).

BYI 02960 SL 200 was applied once to a grassland on 30 June 2010 at nominal rates of 0.51, 1.7, 5.1 and 21 g a.i./ha, respectively, equivalent to typical drift values for different use patterns of the test item. Average application rates per treatment deviated 5% or less from intended rates. A water control treatment and a reference item treatment (lambda-cyhalothrin at a rate of 0.4 L/ha) were run in parallel.

The soil-surface and plant-dwelling arthropod communities were monitored shortly before, one, two, four and eight weeks after application. A broad spectrum of arthropods was sampled with different sampling methods (pitfall trapping, Berlese-Tullgren extraction from weed samples and suction sampling). The trial had a randomized complete block design with 4 replicates / treatment. Each block had six treatment plots. To minimize interference among plots, the trial was laid out in a checkerboard design.

The arthropod community sampled in this study was diverse and typical for grassland vegetation, and representative for an off-crop non-target arthropod community. The timing of the experiment was such that a high number of abundant taxa were present during the sampling period. In addition timing coincided with typical use patterns for the test item.

Application of the insecticidal reference item lambda-cyhalothrin resulted in clear responses at both the arthropod community level and the population level. This was true for taxa and communities collected with all three sample types.

Treatment with the insecticide BYI 02960 SL 200 in an off-field grassland habitat in The Netherlands did not lead to statistically significant effects on prevailing arthropod communities at any of the rates tested up to 21 g a.i./ha.

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At the population level a statistical significant effect occurred for few taxa, but no consistent trend in time or relation to the dose rate was found. It could not be excluded that short term population declines observed only in the sampling moment one week after application for the parasitoids Braconidae (Hymenoptera), and only in the sampling moment two weeks after application for the predatory mite Cunaxidae (Astigmata, Acari) were related to treatment with BYI 02960 SL 200 at the highest rate tested in this study, 21 g a.i./ha. These statistically significant reductions were not seen in lower test rates, nor did they occur on other sampling moments. Due to the very short duration of population declines in both taxa (on one occasion only) the observations are considered biologically irrelevant.

The community NOER (No Observed Effect Rate) of BYI 02960 SL 200 applied in an off-crop grassland in The Netherlands is 21 g a.i./ha. The population NOER is classified as 5.1 g a.i./ha, the population NOEAER (No Observed Ecologically Adverse Effect Rate) is classified as 21 g a.i./ha.

MATERIAL AND METHODS

A. Materials

1. Test material

Test item:	BYI 02960 SL 200
Specification No.:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Batch No.:	2010-001067
Sample description:	TOX 08907-00
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.1% w/w, 201.0 g/L according to certificate of analysis
Density:	1.175 g/mL at 20°C
Stability of test compound:	Approved until 14.06.2012, when stored at 25 ± 5°C

2. Reference item	Karate Zeon (lambda-cyhalothrin)
Batch No.:	BSN902273
MITOX ID	20100625FB01
Nominal content of active ingredient:	Lambda-cyhalothrin: 100 g/L (9.5%)
Stability of test compound:	Approved until June 2012

3. Controls: Water

4. Test site

Test plant:	Uncultivated grass land area with low agricultural input (occasional mowing for hay production).
Location:	[redacted] Netherlands.
Field description:	The meadow was located in an agricultural landscape (meadows, low crops, some bushes). The field was delimited on the western side by a road, on the north- and eastern side by a hedgerow and meadows, on the south side by a dirt road and meadows.

B. Study design and methods

1. In-life dates June 27 to August 30, 2010

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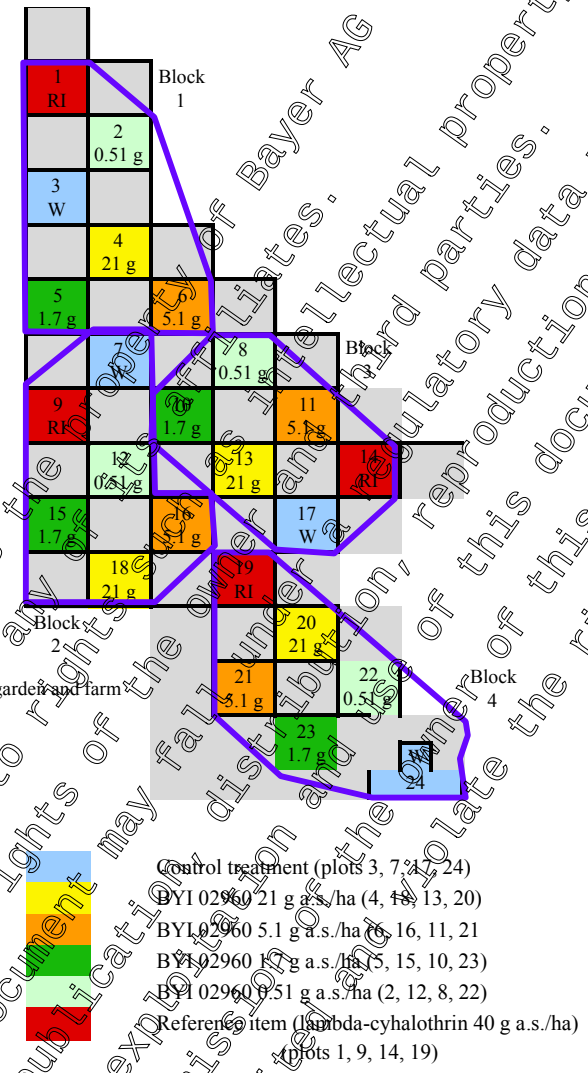
2. Design of biological test

The trial had a randomized complete block design with 4 replicates / treatment. Each block had six treatment plots of 24 x 24 m. To minimize interference among plots, the trial was laid out in a checkerboard design

. Figure Plot layout



BYI 02960 SL 200 was applied once to a grassland on 30 June 2010 at nominal rates of 0.51, 1.7, 5.1 and 21 g a.i./ha, respectively equivalent to typical drift values for different use patterns of the test item. Average application rates per treatment deviated 5% or less from intended rates. A water control treatment and a reference item treatment (lambda-cyhalothrin at a rate of 0.4 L/ha) were run in parallel. Nominal application volumes were 200 L/ha, respectively.

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3. Deviations from conventional test methods for in-crop field studies

Although under directive EC 91/414 off-crop risk to NTA may be assessed using an in-crop exposure scenario it was decided to perform this study in a true off-crop habitat, i.e. a grassland habitat with little agricultural input in The Netherlands used for hay production. The site was surrounded by agricultural fields. This approach had the advantage that the observed response would pertain to a more representative off-crop NTA community, i.e. a community not previously under selection in an agricultural regime. For this reason the study outcome will represent a worst case situation, irrespective of the intended product use.

An additional deviation from the conventional approach under directive EC 91/414 was the choice for a NOER-type study ([redacted] and [redacted], 2007, [redacted] *et al*, 2010), rather than the choice for a study to assess an effect value at certain test rates representative of expected spray drift for a particular product use. Obviously, the choice for a NOER-type approach makes the results applicable to any product use pattern. At the same time the assessment of a NOER/LOER and NOEAER/LOEAER avoids the caveats of assessing the acceptability of certain effect levels at given drift rates. The finding that the NOER may be expected to occur at *x* meter from a treated area will be unambiguously interpretable, as in aquatic ecotoxicology test designs.

4. Observation and measurements

Sampling was confined to the centre of each plot; pitfalls were placed in a central area of approximately 2 x 2 m around the plot centre and suction and weed sampling was done just outside this central area.

The soil-surface and plant-dwelling arthropod communities were monitored shortly before, one, two, four and eight weeks after application. A broad spectrum of arthropods was sampled with different sampling methods.

Pitfall trapping

Taxa at aim: Soil and surface dwelling arthropods, e.g. carabids, spiders and springtails.

Traps were placed in the ground, for a period of three to five days. Sets of two pots were placed on five locations, in a W-configuration (= 10 pots /plot). Each individual trap consisted of a plastic beaker (diameter 9 cm) buried flush with the soil surface, containing ethylene glycol to preserve the arthropods. Each set of two traps was protected from rain and debris by a transparent cover. For each plot all ten pitfalls were pooled into a single sample during collection. Samples were collected by pouring the contents of the beakers into a 2 L pot in the field. Beakers were transferred to the lab, and cleaned with water over a 45 µm sieve. The contents of the sieve were transferred into a labeled 125 or occasionally 250 ml Nalgene jar and 70% ethanol was added to ensure a good preservation of the sample during storage. To prepare for further processing, the material was poured over a stack of sieves. Usually these were sieves which retained fractions > 4 mm, > 2 mm, > 1 mm, > 500 µm, > 300 µm and > 45 µm. After sieving, the material was transferred to Petri dishes for identification and counting. Identifications and counts were carried out under a dissecting microscope.

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For taxa occurring at very high numbers (well over 400 per plot), a subsample of this taxon may have been counted. After homogenising the sample, a fraction of the sample was taken and counted, such that the fraction contained at least 100 specimens of this particular taxon. The total number of specimens of this taxon in the entire sample was then calculated (volume based) and recorded. Taxa with low occurrence in this sample were counted in the complete sample.

Berlese-Tullgren extraction from weed samples

Taxa at aim: Mites and other low mobile small plant inhabiting arthropods.

Weeds were sampled from a predetermined surface of 0.5 m^2 per plot, obtained by collecting two subsamples using an open rectangular mould of 0.25x1 m. All weeds within this mould were cut off just above the soil surface with garden scissors and both subsamples were stored together in a labelled plastic bag and transported to the test facility as soon as possible where they were processed further.

Samples were processed upon arrival at the test facility, in order to preserve both plants and arthropods in a good condition, which is an essential requirement for Berlese-Tullgren extraction. First the sample weights were recorded, then the vegetation samples were placed in a modified Berlese-Tullgren setup. For each plot the sample material was spread out evenly on a coarse grid, above a funnel with a labelled vial attached to the bottom end, containing a 70% ethanol solution. Each funnel was placed under a 40W incandescent light bulb, such that the weeds desiccated gradually in three to seven days, hereby forcing the arthropods to move away from the light and heat source into the vial. Upon the complete desiccation of the sample, the dried weeds were removed and the funnel flushed with a 70% ethanol solution. Then the jars were removed, closed with a lid and stored.

The collected arthropods were inspected under a binocular microscope in ethanol 70% in a U-shaped mite counting channel in a 18 x 8 x 2 cm plexiglas plate. Liquid paraffin was added to the ethanol 70% to separate the arthropods from organic debris. The channel was 2.5 cm wide and 1.2 cm deep. In the middle of the device there was another channel with a depth of approximately 0.5 cm and a width of approximately 1.5 cm. The arthropods were in the middle channel. The lower channel served as an overflow. Measures given are approximate. The device containing the sample is appropriate for inspection with a microscope with a magnification of 40.

In this study Oribatida, Phytoseiidae and other Mesostigmata were considered key groups. These taxa and some other abundant taxa were identified further to the lowest possible taxonomic level. Hereto a maximum of 15 impartially chosen specimens (for each plot) were mounted for microscopic examination. This was done only for the pre-application sample and the sample taken 1 month after application (sample 1 and 4, respectively). Mites were identified using extensive keys and original species descriptions available at the MITOX taxonomy laboratory.

Suction sampling.

Taxa at aim: Small, low and highly mobile plant inhabiting arthropods (e.g. micro-hymenoptera).

Suction sampling was performed over a larger surface around the central 2 x 2 m plot centre, but avoiding the outer 5 m zones of the plots.

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Sampling was done with a D-vac machine (model 24, D-Vac Company) which draws insects into a net, placed in the machine tube mouth, through suction. During a period of 4 minutes the mouth of the aspirator was moved over and through the weeds around the plot center, approximately 1 minute per plot side, hereby collecting the present plant-dwelling walking and flying arthropods. The time was monitored with a stopwatch. After 4 minutes ethyl acetate was added to the sample. Subsequently the nets were closed with a knot, put into a labelled plastic bag and transported to the test facility for further processing.

The samples were transferred from the nets into labelled 250 mL jars (at maximum one or two days after collection). When samples were not transferred immediately after collection, they were stored in a fridge at about 5 ºC. Transferring was achieved by holding the collection net inside-out above a large funnel, with the jar fitted on its tip, and brushing the contents of the net downwards with a brush. Once the net was visually clean from debris/arthropods the funnel was flushed with 70% ethanol and the jar filled up to preserve the sample during storage.

Table 10.5.4- 1: Time schedule of sampling

Sample no.	Days after application	Pitfall *placed*	Pitfall *collected*	Weed	Suction
1	0/-1	27-Jun-10	30-Jun-10	29-Jun-10	29-Jun-10
Application 30-Jun-10	**0**	**-**	**-**	**-**	**-**
2	7	2-Jul-10	7-Jul-10	7-Jul-10	7-Jul-10
3	14	9-Jul-10	14-Jul-10	14-Jul-10	14-Jul-10
4	28	23-Jul-10	28-Jul-10	28-Jul-10	28-Jul-10
5	56	20-Aug-10	25-Aug-10	25-Aug-10	25-Aug-10

5. Statistical analysis

Effects on ground dwelling- and plant inhabiting arthropod communities were examined using multivariate analysis techniques (ordination) applied to the entire dataset (for each sampling method separately, and with data from all sampling techniques pooled). The advantage of multivariate methods over univariate methods is that they use and summarize all information on the investigated populations simultaneously, and in doing so they evaluate the effects of pesticide treatments at the community level. Effects of the test item treatments at the community level were expressed relative to the control using the Principal Response Curve method. This form of canonical ordination involves the inclusion of time as a covariable in the analysis. This technique is especially suitable for ecotoxicological studies where treatment is the explanatory variable, and species compositions are the response variables.

In addition to the community level analyses, effects on individual taxa (population level) were also assessed using univariate statistics. Due to differences in species abundances and/or due to restrictions in the taxonomic level used for identification, univariate analyses were performed at different taxonomic levels. Only taxa that occurred at densities above 10 (total per water control treatment) were considered. If taxonomically relevant, taxa were analyzed pooled if densities of individual taxa were too low for evaluation. The statistical significance of among treatment differences in population density was determined separately for all time points using the nonparametric Mann-Whitney U test.

Statistical significances were in principal considered at an alpha level of 5%. Statistical significances at an alpha level of 10% were also indicated as additional information to evaluate potential trends.

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Multivariate analyses were performed with the computer program CANOCO for Windows version 4.5 (2002). Data were imported from Excel to CANOCO with WcanoImp 1.0, a utility of CANOCO 4.5. Principal Response Curve graphs were made in Excel. Monte Carlo Permutation tests to determine the significance of the first and the second ordination axis in the community analyses were performed in CANOCO 4.5 as part of the Ordination/Redundancy Analysis (RDA).

Univariate tests were done with SPSS 18 for the Macintosh.

Expert judgement:

In addition to statistical analyses, biological information about arthropod taxa can also be incorporated in final effect classifications (e.g. strong aggregation behaviour inducing additional variation, high mobility tendency, etc.). Furthermore, pre-application circumstances and data from all test rates can be considered in final decisions regarding effect levels at individual test rates and individual taxa. Expert judgement is needed to determine whether an observed difference from the control is an artefact or related to the test item treatment.

Effect classes:

Effects are classified according to [redacted] *et al.* (2010). Different effect classes are listed below. For this study with a total post-application sampling period of two months only effect classes 1, 2, 3 and 8 are applicable. According to [redacted] et al. (2010), recovery should to be demonstrated on two consecutive sampling moments. Due to the 4 week interval between the last two sampling moments this would result in a very conservative effect classification. Effect class 3 was therefore subdivided in class 3a (no longer statistically significant on the last two sampling dates) and class 3b (no longer statistically significant on the last sampling date).

Table 10.5.4- 2: Effect classes according to [redacted] *et al.* (2010)

Effect class	Description	Criteria
1	Effects could not be demonstrated (NOER)	• No (statistically significant) effects observed as a result of the treatment • Observed differences between treatment and controls show no clear causal relationship
2	Slight and transient effects	• Quantitatively restricted response of one or a few taxa and only observed on one sampling occasion
3	Pronounced short term effects; recovery within two months after first application	• Clear response of taxa, but full recovery within two months after the first application • Effects observed at two or more sampling instances
4	Pronounced effects; recovery within four months after first application	• Clear response of taxa, effects last longer than two months but full recovery within four months after the first application • Effects observed at two or more sampling instances
5	Pronounced effects; recovery within eight months after first application	• Clear response of taxa, effects last longer than four months but full recovery within eight months after the first application • Effects observed at two or more sampling instances
6	Pronounced effects; full recovery one year after first application	• Clear response of taxa, effects last longer than eight months but full recovery within one year after first application • Effects observed at two or more sampling instances
7	Pronounced effects; full recovery more than one year after first application	• Clear response of taxa, effects last longer than twelve months after the first application but full recovery found within the study period • Effects observed at two or more sampling instances
8	Pronounced effects; no recovery within the study period	• Clear response of taxa, no recovery within the duration of the study • Effects observed at two or more sampling instances

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Definitions:

NOER: no observed effect rate. (As described for effect class 1 in the table above)

NOEAER: no observed ecologically adverse effect rate. The highest test rate at which recovery within 2 months occurred (as described for effect class 3 in the table above)

LOEAER: lowest observed ecologically adverse effect rate. The lowest test rate at which no recovery within 2 months occurred (as described for effect class 8 in the table above)

RESULTS AND DISCUSSION

A. Suitability of the current test method

NTA fauna

The number of taxa occurring at sufficiently high numbers to allow for a population level analysis (72 taxa) was higher than the number of taxa usually evaluated in studies performed in commercial agricultural settings. This made the study more powerful than a conventional in-crop study.

Sampling techniques

By using three different collecting methods (pitfall, Berlese Tullgren extraction from weed, and suction sampling techniques) all strata of the arthropod community occurring in grasslands were comprehensively sampled (ground dwelling arthropods and weed inhabiting arthropods).

Plot size

No recovery was seen for many taxa in the positive reference treatment, indicating that for the experimental period chosen, the plot size was adequate to demonstrate persistent treatment related effects.

It is concluded that the test method presented in this study accurately examines potential risks for NTA fauna in true and representative off-crop habitats under a realistic worst-case scenario.

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B. Application rates

SPRAYER CALIBRATION

application date: 30 June 2010

run no.	calibration date	spraying time (seconds)	total volume (L)	rate (L/min)
1	30-Jun-10	121	50.00	24.79
2	30-Jun-10	120	49.00	24.50
3	30-Jun-10	121	50.00	24.79
average		120.67	49.67	24.70
target		120	50.00	24.00
deviation		0.56%	-0.67%	2.90%

spray pressure:	2 bar
spray volume:	200 L/ha
nozzle type:	Hardi Injet blue 03
spray boom length:	12 m (12.5 m spray width)
total number of nozzles:	25 (50 cm apart)
Target application rate:	200 L/ha
Target volume per plot:	12.0 L

PREPARATION OF TEST SOLUTIONS

treatment	plots treated	water volume (L)	test item quantity (g product)	time solution prepared	Content a.i: (BYI)	17.1% w/w
Water	3, 7, 17, 24	nr	-	n.r.		
21 g a.s./ha	4, 13, 18, 20	50.23	30.67	12:30	a.s. = BYI 02960	
5.1 g a.s./ha	6, 11, 16, 21	50.09	7.43	12:03	a.s. = BYI 02960	
1.7 g a.s./ha	5, 10, 15, 23	49.98	2.50	11:35	a.s. = BYI 02960	
0.51 g a.s./ha	2, 8, 12, 22	50.08	0.74	10:45	a.s. = BYI 02960	
RI (40 g a.s./ha)	1, 9, 14, 19	50.05	109.59	13:00	RI = Reference Item = lambda-cyhalothrin	

APPLIED VOLUMES AND RATES

treatment	plots treated	treated plot surface (m^2)*	total volume applied (L)	actual application volume (L/ha)	standard deviation (L)	deviation from target volume (%)	actual application rate (g a.s./ha)	standard deviation (g)	deviation from target rate (%)
Water	3	600	13	217		8.33%			
Water	7	600	13	217		8.33%			
Water	17	600	13	217		8.33%			
Water	24	600	13	217		8.33%			
		2400	**average**	**217**	**0**	**8.33%**			
21 g a.s./ha	4	600	11	183		-8.33%	19.14		-8.8%
21 g a.s./ha	13	600	13	217		8.33%	22.62		7.7%
21 g a.s./ha	18	600	12	200		0.00%	20.88		-0.6%
21 g a.s./ha	20	600	12	200		0.00%	20.88		-0.6%
			average	**200**	**14**	**0.00%**	**20.88**	**1.42**	**-0.6%**
5.1 g a.s./ha	6	600	12	200		0.00%	5.07		-0.5%
5.1 g a.s./ha	11	600	12	200		0.00%	5.07		-0.5%
5.1 g a.s./ha	16	600	12	200		0.00%	5.07		-0.5%
5.1 g a.s./ha	21	600	14	233		16.67%	5.92		16.0%
			average	**208**	**17**	**4.17%**	**5.28**	**0.42**	**3.6%**
1.7 g a.s./ha	5	600	12	200		0.00%	1.71		0.6%
1.7 g a.s./ha	10	600	13	217		8.33%	1.85		9.0%
1.7 g a.s./ha	15	600	13	217		8.33%	1.85		9.0%
1.7 g a.s./ha	23	600	12	200		0.00%	1.71		0.6%
			average	**211**	**10**	**5.56%**	**1.81**	**0.08**	**6.2%**
0.51 g a.s./ha	2	600	13	217		8.33%	0.55		7.3%
0.51 g a.s./ha	8	600	13	217		8.33%	0.55		7.3%
0.51 g a.s./ha	12	600	12	200		0.00%	0.51		-0.9%
0.51 g a.s./ha	22	600	14	233		16.67%	0.59		15.6%
			average	**217**	**14**	**8.33%**	**0.55**	**0.03**	**7.3%**
RI	1	600	14	233		16.67%	46.66		16.7%
RI	9	600	12	200		0.00%	39.99		0.0%
RI	14	600	13	217		8.33%	43.33		8.3%
RI	19	600	11	183		-8.33%	36.66		-8.3%
			average	**208**	**22**	**4.17%**	41.66	**4.30**	**4.2%**

* Plot size was 24*24=576 m^2. However, with a boom length of 12 m, the width of the treated area was 2 x 12.5 = 25 m, hence 0.5 m of the buffer areas were sprayed additionally. Due to the checkerboard design of the plot lay-out, no overspray on other treatment plots occurred. The treated area per plot was therefore 24*25=600 m^2.

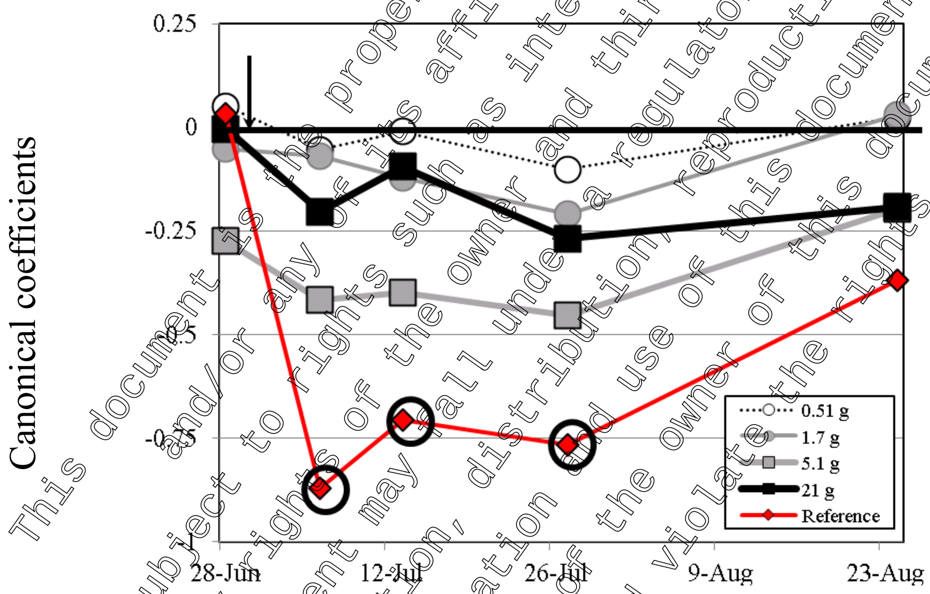
C. Biological Findings

Community level effects

Treatment with the insecticide BYI 02960 SL 200 in an off-field grassland habitat in The Netherlands did not lead to statistically significant effects on prevailing arthropod communities at any of the rates tested up to 21 g a.i./ha. Visual inspection of the Principal Response Curve (PRC) (see figure below) confirmed this finding. Overall lower canonical values for the 5.1 g a.i./ha rate were due to lower starting densities and were therefore unrelated to treatment. Examination of community responses obtained from separate datasets (different sampling methods) did not reveal any treatment related adverse effect on arthropod communities.

Figure: **Principle Response Curve (first ordination axis entire dataset)**

Test- and reference items were analyzed separately but for comparison plotted in one graph. PRC analyses comprised data from weed (W)-, pitfall (P)- and suction (S) samples. Encircled data points are statistically significantly different from the control (Monte-Carlo Permutation test, alpha = 0.05).



Population level effects

At the population level a statistical significantly difference from the control incidentally occurred for few taxa, but no consistent trends in time or relation to the dose rate was found. It could not be excluded that short term population declines observed only in the sampling moment one week after application for the parasitoids *Braconidae* (Hymenoptera), and only in the sampling moment two weeks after application for the predatory mites Cunaxidae (Astigmata, Acari) were related to treatment with BYI 02960 SL 200 at the highest rate tested in this study, 21 g a.i./ha. These statistically significant reductions were not seen in lower test rates, nor did they occur on other sampling moments. Due to the very short duration of population declines in both taxa (on one occasion only), the observations are considered biologically irrelevant.

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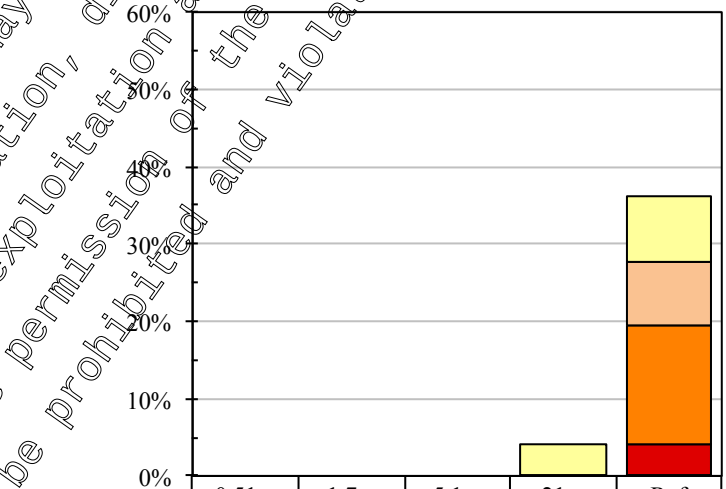
Summary population level effects:

Effect classification:		
one occasion	Clear adverse treatment related effect but observed only on one occasion	2
< 2 months (a)	no longer statistically significant on the last two sampling moments	3a
< 2 months (b)	no longer statistically significant on the the last sampling moment	3b
> 2 months	No recovery within the study period (= 2 months)	8
	Based on 10% significance level and visual consideration of trends	2
	Based on 5% significance level and visual consideration of trends	

Order	Taxon	method	0.51 g	1.7 g	5.1 g	21 g	Ref
ACARI	Phytoseiidae (female)	W					3b
	Gamasida other (female)	W					3b
	Gamasida Nymph and male	W					3b
	Cunaxidae	W				2	3b
	Tydeoidea	W					3b
HOMOPTERA	Cicadellidae (juveniles)	P					8
COLEOPTERA	Tachyporinae	P					3a
ARANEAE	*Phrurolithus* (adult)	P					3b
	Pardosa (adult)	P					3a
	Pachygnatha	P					3
THYSANOPTERA	Thysanoptera (juveniles)	S					3b
	Thysanoptera (adults)	S					3a
HOMOPTERA	Aphidoidea	S					3a
	Cicadellidae (juveniles)	S					3b
HETEROPTERA	Miridae (adults)	S					3b
	Miridae (juveniles)	S					2
DIPTERA	Agromyzidae	S					3b
	Chloropidae	S					3a
	Acalyptrata (others)	S					2
HYMENOPTERA	Formicidae	S					3a
	Alysiinae	S				2	2
	Braconidae (other)	S				2	2
COLEOPTERA	Aleocharinae	S					8
ARANEAE	*Oxyticus* (juvenile)	S					3b
	Pachygnatha (juvenile)	S					2
	Araneidae (juvenile)	S					8

Total number of taxa evaluated: 72

W=weed samples/Berlese extraction
P=pitfall
S-suction



	0.51 g	1.7 g	5.1 g	21 g	Ref
one occasion	0.0%	0.0%	0.0%	4.2%	8.3%
< 2 months (a)	0.0%	0.0%	0.0%	0.0%	8.3%
< 2 months (b)	0.0%	0.0%	0.0%	0.0%	15.3%
> 2 months	0.0%	0.0%	0.0%	0.0%	4.2%

According to De Jong *et al.* (2010), recovery should be demonstrated in two consecutive sampling moments. Due to the 4 week interval between the last two sampling moments this would result in a very conservative effect classification. Effect class 3 was therefore subdivided in class 3a (no longer statistically significant on the last two sampling moments) and class 3b (no longer statistically significant on the last sampling moment).

Only taxa that were adversely affected by the test item or by the reference item are shown.

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Summary effect classifications

Table 10.5.4- 3: Effect classifications of BYI 02960 SL 200 rates

Community level effects (PRC/Monte-Carlo; 5% alpha level)	**0.51 g a.s./ha**	**1.7 g a.s./ha**	**5.1 g a.s./ha**	**21 g a.s./ha**
		Effect class		
	1	1	1	1
Conclusion				**Community NOER**

Population level effects (Mann-Whitney U test; 5% alpha level)	**0.51 g a.s./ha**	**1.7 g a.s./ha**	**5.1 g a.s./ha**	**21 g a.s./ha**
		Effect class		
Cunaxidae (Prostigmata, Acari)	1	1	1	2
Braconidae (Hymenoptera)	1	1	1	2
Conclusion			**Population NOER**	**Population NOEAER**

NOER:	No Observed Effect Rate (no statistically significant differences compared to control)
NOEAER:	No Observed Ecologically Adverse Effect Rate (at least 1 taxon with effect class 2 or 3, i.e. clear response to treatment but with recovery within 2 months after application)

D. Biological Endpoints Derived

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

NOER (community)	21 g a.i./ha in an off-crop grassland
NOER (population)	5.1 g a.i./ha in an off-crop grassland
NOEAER (population)	21 g a.i./ha in an off-crop grassland

CONCLUSION

The community NOER (No Observed Effect Rate) of BYI 02960 SL 200 applied in an off-crop grassland in The Netherlands is 21 g a.i./ha.

The predatory mites Cunaxidae and the hymenopteran parasitoids Braconidae were adversely affected only on one sampling moment shortly after application in the highest test rate. Both taxa recovered within one week. The rate of 5.1 g a.i./ha is classified as the population NOER (No Observed Effect Rate) and the rate of 21 g a.i./ha is classified as the population NOEAER (No Observed Ecologically Adverse Effect Rate) of BYI 02960 SL 200.

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Report:	**KIIIA1 10.5.4/02; [redacted] S., [redacted] F.; 2012b**
Title:	A field study to assess the effects of BYI 02960 (SL 200 g/L) on the non-target, surface- and plant-dwelling arthropod fauna of a grassland habitat (off-crop) in SW France during summer
Report No:	B153FFN
Document No:	M-425080-01-2
Guidelines:	**IOBC ([redacted], 1992), ANONYMOUS (1992), [redacted] (1998), IOBC, BART AND EPPO JOINT INITIATIVE ([redacted] ET AL., 2000, 2001), [redacted] ET AL., 2010**
Deviations from guideline:	**Use of true off-crop habitat (grassland habitat with little agricultural input) representing a realistic worst-case scenario** **Use of NOER-type study (No Observed Effect Rate), making the results applicable to any product use pattern.**
GLP:	**Yes (certified laboratory)**

Executive Summary

BYI 02960 SL 200 is an insecticide with a wide range of uses. This field study was designed to assess the potential adverse effects on Non-Target Arthropods (NTA) in off-crop habitats that might occur at various distances from a treated area for current and future use pattern of the test item. The study was set up to enable an assessment of community- and population level ecotoxicological standards, in particular the NOER (No Observed Effect Rate), the NOEAER and the LOEAER (No and Lowest Observed Ecologically Adverse Effect Rate, respectively).

BYI 02960 SL 200 was applied once to a grassland on 20 July 2010 at nominal rates of 0.51, 1.7, 5.1 and 21 g a.i./ha, respectively, equivalent to typical drift values for different use patterns of the test item. Average application rates per treatment deviated 5% or less from intended rates. A water control treatment and a reference item treatment (lambda-cyhalothrin at a rate of 0.4 L/ha) were run in parallel.

The soil-surface and plant-dwelling arthropod communities were monitored shortly before, one, two, four and eight weeks after application. A broad spectrum of arthropods was sampled with different sampling methods (pitfall trapping, Berlese-Tullgren extraction from weed samples and suction sampling). The trial had a randomized complete block design with 4 replicates / treatment. Each block had six treatment plots. To minimize interference among plots, the trial was laid out in a checkerboard design.

The arthropod community sampled in this study was diverse and typical for grassland vegetation, and representative for an off-crop non-target arthropod community. The timing of the experiment was such that a high number of abundant taxa were present during the sampling period. In addition, timing coincided with typical use patterns for the test item.

Application of the insecticidal reference item lambda-cyhalothrin resulted in clear responses at both the arthropod community level and the population level. This was true for taxa and communities collected with all three sample types.

Treatment with the insecticide BYI 02960 SL 200 in an off-field grassland habitat did not lead to statistically significant effects on prevailing arthropod communities at any of the rates tested up to 21 g a.i./ha. Visual inspection of the PRC graph (Principal Response Curve) showed differences between the highest two test item rates and the control which were of small magnitude and short duration, and not statistically significant at any sampling moment.

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Seventy-nine taxa were abundant enough for population level analysis. Three phytophagous taxa showed statistically significant adverse response patterns that were considered related to the test item treatment (based on magnitude and/or duration in relation to dose, timing).

Aphidoidea (Homoptera) collected with suction sampling were reduced compared to the control on three consecutive sampling moments after application in the highest test item rate (21 g a.i./ha). The effect was statistically significant at an alpha level of 0.05 only on the sampling moment one month after application. On the last sampling moment (two months after applications) densities were similar to control densities.

Furthermore the juvenile *Cicadellidae* (Homoptera) collected with suction sampling were statistically significantly reduced compared to the control one and two weeks after application in the highest test item rate (21 g a.i./ha). On the last two sampling moments the differences to the control were no longer statistically significant. Densities in the 5.1 g a.i./ha rate were statistically significantly reduced on the sampling moment immediately after treatment. During the remainder of the sampling period densities were similar to the control in this test item treatment.

Alticinae (Chrysomelidae, Coleoptera, suction sampling) were statistically significantly reduced in the 5.1 g and 21 g a.i./ha rate one or two weeks after application. Differences compared to the control were not statistically significant on the last two sampling moments.

For few other taxa reductions compared to the control occurred incidentally, but no consistent trend in time or relation to the dose rate was found.

The community NOER (No Observed Effect Rate) of BYI 02960 SL 200 applied in an off-crop grassland in South-West France is 21 g a.i./ha. The population NOER is classified as 1.7 g a.i./ha, the population NOEAER (No Observed Ecologically Adverse Effect Rate) is classified as 21 g a.i./ha.

MATERIAL AND METHODS

A. Materials

1. Test material

Test item:	BYI 02960 SL 200
Specification No.:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Batch No.:	2010-001067
Sample description:	TOX08907-00
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.1% w/w, 201.0 g/L according to certificate of analysis
Density:	1.175 g/mL at 2ß°C
Stability of test compound:	Expiry date: 14.06.2012, when stored at 25 ± 5°C

2. Reference item	Karate Zeon (lambda-cyhalothrin)
Batch No.:	BSN9H2472
MITOX ID:	20100505JR01
Nominal content of active ingredient:	Lambda-cyhalothrin: 100 g/L (9.5%)
Stability of test compound:	Approved until 2011 May

3. Controls: Water

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4. Test site

Test plant:	Uncultivated grass land area with low agricultural input (occasional mowing for hay production). No pesticides were used for the last 5 years preceding this study
Location:	[redacted] France
Field description:	The meadow was delimited on the northeastern side by a road, on the northwestern side by a house and gardens, at the southeast and southwest side by vineyards. It was gently sloping down towards the southwest.

B. Study design and methods

1. In life dates July 13 to September 18, 2010

2. Design of biological test

The trial had a randomized complete block design with 4 replicates / treatment. Each block had six treatment plots of 22 x 22 m. To minimize interference among plots, the trial was laid out in a checkerboard design.

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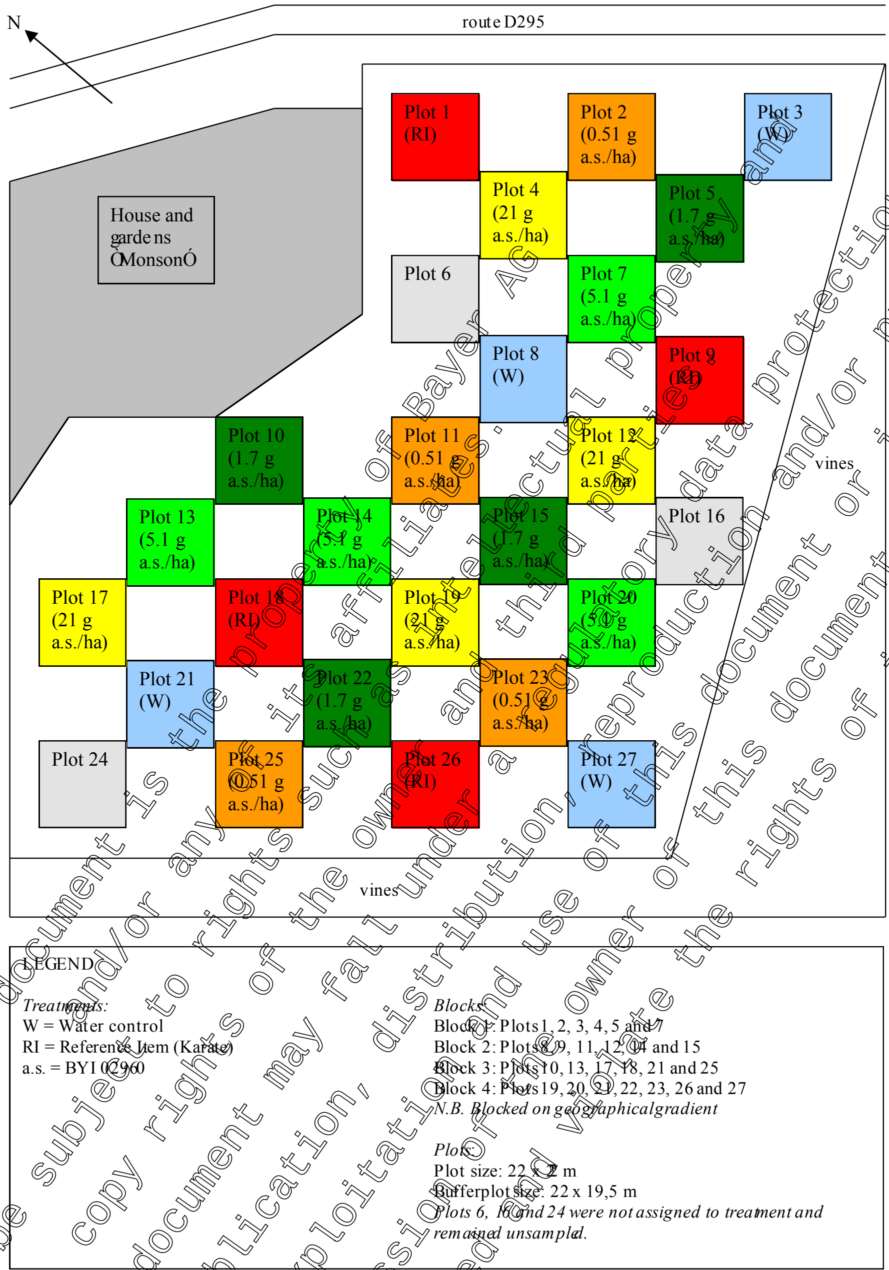
Figure **Plot layout**



BYI 02960 SL 200 was applied once to a grassland on 20 July 2010 at nominal rates of 0.51, 1.7, 5.1 and 21 g a.i./ha, respectively, equivalent to typical drift values for different use patterns of the test item. Average application rates per treatment deviated 5% or less from intended rates. A water control treatment and a reference item treatment (lambda-cyhalothrin at a rate of 0.4 L/ha) were run in parallel. Nominal application volumes were 200 L/ha.

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3. Deviations from conventional test methods for in-crop field studies

Although under regulation (EC) 1107/2009 off-crop risk to NTA may be assessed using an in-crop exposure scenario it was decided to perform this study in a true off-crop habitat, i.e. a grassland habitat with little agricultural input in The Netherlands used for hay production. The site was surrounded by agricultural fields. This approach had the advantage that the observed response would pertain to a more representative off-crop NTA community, i.e. a community not previously under selection in an agricultural regime. For this reason the study outcome will represent a worst case situation, irrespective of the intended product use.

An additional deviation from the conventional approach under Regulation (EC) 1107/2009 was the choice for a NOER-type study rather than the choice for a study to assess an effect value at certain test rates representative of expected spray drift for a particular product use. Obviously, the choice for a NOER-type approach makes the results applicable to any product use pattern. At the same time the assessment of a NOER/LOER and NOEAER/LOEAER avoids the caveats of assessing the acceptability of certain effect levels at given drift rates. The finding that the NOER may be expected to occur at *x* meter from a treated area will be unambiguously interpretable, as in aquatic ecotoxicology test designs.

4. Observation and measurements

Sampling was confined to the centre of each plot; pitfalls were placed in a central area of approximately 2 x 2 m around the plot centre and suction and weed sampling was done just outside this central area.

The soil-surface and plant-dwelling arthropod communities were monitored shortly before, one, two, four and eight weeks after application. A broad spectrum of arthropods was sampled with different sampling methods.

Pitfall trapping

Taxa at aim: Soil and surface dwelling arthropods, e.g. carabids, spiders and springtails.

Traps were placed in the ground, for a period of four to seven days. Sets of two pots were placed on five locations, in a W-configuration (= 10 pots /plot). Each individual trap consisted of a plastic beaker (diameter 9 cm) buried flush with the soil surface, containing ethylene glycol to preserve the arthropods. Each set of two traps was protected from rain and debris by a transparent cover. For each plot all ten pitfalls were pooled into a single sample during collection. Samples were collected by pouring the contents of the beakers over a sieve (mesh size 90 µm). The contents of the sieve were transferred into a labelled 125 ml Nalgene jar and 70% ethanol was added to ensure a good preservation of the sample during storage. To prepare for further processing, the material was poured over a stack of sieves. Usually these were sieves which retained fractions > 4 mm, > 2 mm, > 1 mm, > 500 µm, > 300 µm and > 45 µm. After sieving, the material was transferred to Petri dishes for identification and counting. Identifications and counts were carried out under a dissecting microscope.

For taxa occurring at very high numbers (well over 400 per plot), a subsample of this taxon may have been counted. After homogenising the sample, a fraction of the sample was taken and counted, such that the fraction contained at least 100 specimens of this particular taxon. The total number of specimens of this taxon in the entire sample was then calculated (volume based) and recorded. Taxa with low occurrence in this sample were counted in the complete sample.

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Berlese-Tullgren extraction from weed samples

Taxa at aim: Mites and other low mobile small plant inhabiting arthropods.

Weeds were sampled from a predetermined surface of 0.5 m^2 per plot, obtained by collecting two subsamples using an open rectangular mould of 0.25x1 m. All weeds within this mould were cut off just above the soil surface with a motorized trimmer and both subsamples were stored together in a labelled plastic bag and transported to the test facility as soon as possible where they were processed further.

Samples were processed upon arrival at the test facility, in order to preserve both plants and arthropods in a good condition, which is an essential requirement for Berlese-Tullgren extraction. First the sample weights were recorded, then the vegetation samples were placed in a modified Berlese-Tullgren setup. For each plot the sample material was spread out evenly on a coarse grid, above a funnel with a labelled vial attached to the bottom end, containing a 70% ethanol solution. Each funnel was placed under a 40W incandescent light bulb, such that the weeds desiccated gradually in three to seven days, hereby forcing the arthropods to move away from the light and heat source into the vial. Upon the complete desiccation of the sample, the dried weeds were removed and the funnel flushed with a 70% ethanol solution. Then the jars were removed, closed with a lid and stored.

The collected arthropods were inspected under a binocular microscope in ethanol 70% in a U-shaped mite counting channel in a 18 x 8 x 2 cm plexiglas plate. Liquid paraffin was added to the ethanol 70% to separate the arthropods from organic debris. The channel was 2.5 cm wide and 1.2 cm deep. In the middle of the device there was another channel with a depth of approximately 0.5 cm and a width of approximately 1.5 cm. The arthropods were in the middle channel. The lower channel served as an overflow. Measures given are approximate. The device containing the sample is appropriate for inspection with a microscope with a magnification of 40.

In this study Oribatida, Phytoseiidae and other Mesostigmata were considered key groups. These taxa and some other abundant taxa were identified further to the lowest possible taxonomic level. Hereto a minimum of 15 impartially chosen specimens (total from all plots) were mounted for microscopic examination. This was done only for the pre-application sample and the sample taken 1 month after application (sample 1 and 4, respectively).

Suction sampling.

Taxa at aim: Small, low and highly mobile plant inhabiting arthropods (e.g. micro-hymenoptera).

Suction sampling was performed over a larger surface around the central 2 x 2 m plot centre, but avoiding the outer 5 m zones of the plots.

Sampling was done with a D-vac machine (model 24, D-Vac Company) which draws insects into a net, placed in the machine tube mouth, through suction. During a period of 4 minutes the mouth of the aspirator was moved over and through the weeds around the plot center, approximately 1 minute per plot side, hereby collecting the present plant-dwelling walking and flying arthropods. The time was monitored with a stopwatch. After 4 minutes ethyl acetate was added to the sample. Subsequently the nets were closed with a knot, put into a labelled plastic bag and transported to the test facility for further processing.

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The samples were transferred from the nets into labelled 250 mL jars (at maximum one or two days after collection). When samples were not transferred immediately after collection, they were stored in a fridge at about 5 ºC. Transferring was achieved by holding the collection net inside-out above a large funnel, with the jar fitted on its tip, and brushing the contents of the net downwards with a brush. Once the net was visually clean from debris/arthropods the funnel was flushed with 70% ethanol and the jar filled up to preserve the sample during storage.

Table 10.5.4- 4: Time schedule of sampling

Sample no.	DAA *Note 1*	Pitfall *placed*	Pitfall *collected*	Weed *collected*	Suction *collected*
1	-6 to -3	13 July 2010	17 July 2010	14 July 2010	14 July 2010
Application (20 July 2010)	**0**	-	-	-	-
2	7	23 July 2010	27 July 2010	27 July 2010	27 July 2010
3	14	27 July 2010	3 August 2010	3 August 2010	3 August 2010
4	28	10 August 2010	17 August 2010	17 August 2010	17 August 2010
5	56	10 Sept 2010	14 Sept 2010	14 Sept 2010	14 Sept 2010

Note 1: Days After Application (DAA) refers to the collection dates.

5. Statistical analysis

Effects on ground dwelling- and plant inhabiting arthropod communities were examined using multivariate analysis techniques (ordination) applied to the entire dataset (for each sampling method separately, and with data from all sampling techniques pooled). The advantage of multivariate methods over univariate methods is that they use and summarize all information on the investigated populations simultaneously, and in doing so they evaluate the effects of pesticide treatments at the community level. Effects of the test item treatments at the community level were expressed relative to the control using the Principal Response Curve method. This form of canonical ordination involves the inclusion of time as a covariable in the analysis. This technique is especially suitable for ecotoxicological studies where treatment is the explanatory variable, and species compositions are the response variables.

In addition to the community level analyses, effects on individual taxa (population level) were also assessed using univariate statistics. Due to differences in species abundances and/or due to restrictions in the taxonomic level used for identification, univariate analyses were performed at different taxonomic levels. Only taxa that occurred at densities above 10 (total per water control treatment) were considered. If taxonomically relevant, taxa were analyzed pooled if densities of individual taxa were too low for evaluation. The statistical significance of among treatment differences in population density was determined separately for all time points using the nonparametric Mann-Whitney U test.

Statistical significances were in principal considered at an alpha level of 5%. Statistical significances at an alpha level of 10% were also indicated as additional information to evaluate potential trends.

Multivariate analyses were performed with the computer program CANOCO for Windows version 4.5 (2002). Data were imported from Excel to CANOCO with WcanoImp 1.0, a utility of CANOCO 4.5. Principal Response Curve graphs were made in Excel. Monte Carlo Permutation tests to determine the significance of the first and the second ordination axis in the community analyses were performed in CANOCO 4.5 as part of the Ordination/Redundancy Analysis (RDA).

Univariate tests were done with SPSS 18 for the Macintosh.

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Expert judgement:

In addition to statistical analyses, biological information about arthropod taxa can also be incorporated in final effect classifications (e.g. strong aggregation behaviour inducing additional variation, high mobility tendency, etc.). Furthermore, pre-application circumstances and data from all test rates can be considered in final decisions regarding effect levels at individual test rates and individual taxa. Expert judgement is needed to determine whether an observed difference from the control is an artifact or related to the test item treatment.

Effect classes:

Effects are classified according to [redacted] *et al*. (2010). Different effect classes are listed below. For this study with a total post-application sampling period of two months only effect classes 1, 2, 3 and 8 are applicable. According to [redacted] et al. (2010), recovery should to be demonstrated on two consecutive sampling moments. Due to the 4 weeks interval between the last two sampling moments this would result in a very conservative effect classification. Effect class 3 was therefore subdivided in class 3a (no longer statistically significant on the last two sampling dates) and class 3b (no longer statistically significant on the last sampling date).

Table 10.5.4- 5: Effect classes according to [redacted] *et al.* (2010)

Effect class	Description	Criteria
1	Effects could not be demonstrated (NOER)	• No (statistically significant) effects observed as a result of the treatment • Observed differences between treatment and controls show no clear causal relationship
2	Slight and transient effects	• Quantitatively restricted response of one or a few taxa and only observed on one sampling occasion
3	Pronounced short term effects; recovery within two months after first application	• Clear response of taxa, but full recovery within two months after the first application • Effects observed at two or more sampling instances
4	Pronounced effects; recovery within four months after first application	• Clear response of taxa, effects last longer than two months but full recovery within four months after the first application • Effects observed at two or more sampling instances
5	Pronounced effects; recovery within eight months after first application	• Clear response of taxa, effects last longer than four months but full recovery within eight months after the first application • Effects observed at two or more sampling instances
6	Pronounced effects; full recovery one year after first application	• Clear response of taxa, effects last longer than eight months but full recovery within one year after first application • Effects observed at two or more sampling instances
7	Pronounced effects; full recovery more than one year after first application	• Clear response of taxa, effects last longer than twelve months after the first application but full recovery found within the study period • Effects observed at two or more sampling instances
8	Pronounced effects; no recovery within the study period	• Clear response of taxa, no recovery within the duration of the study • Effects observed at two or more sampling instances

Definitions:

NOER: no observed effect rate. (As described for effect class 1 in the table above)

NOEAER: no observed ecologically adverse effect rate. The highest test rate at which recovery within 2 months occurred (as described for effect class 3 in the table above)

LOEAER: lowest observed ecologically adverse effect rate. The lowest test rate at which no recovery within 2 months occurred (as described for effect class 8 in the table above)

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RESULTS AND DISCUSSION

A. Suitability of the current test method

NTA fauna

The number of taxa occurring at sufficiently high numbers to allow for a population level analysis (79 taxa) was higher than the number of taxa usually evaluated in studies performed in commercial agricultural settings. This made the study more powerful than a conventional in-crop study.

Sampling techniques

By using three different collecting methods (pitfall, Berlese Tullgren extraction from weed, and suction sampling techniques) all strata of the arthropod community occurring in grasslands were comprehensively sampled (ground dwelling arthropods and weed inhabiting arthropods).

Plot size

No recovery was seen for many taxa in the positive reference treatment, indicating that for the experimental period chosen, the plot size was adequate to demonstrate persistent treatment related effects.

It is concluded that the test method presented in this study accurately examines potential risks for NTA fauna in true and representative off-crop habitats under a realistic worst-case scenario.

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B. Application rates

SPRAYER CALIBRATION

application date: 20 July 2010

run no.	calibration date	tractor speed (seconds/50m)	vol. left (L/50m)	vol. right (L/50m)	total volume (L/50m)	rate (L/min)
1	20-Jul-10	37	4.12	4.12	8.24	13.36
2	20-Jul-10	37	3.83	4.21	8.04	13.04
3	20-Jul-10	36	4.38	3.85	8.23	13.72
4	20-Jul-10	37	4.24	3.79	8.03	13.02
5	20-Jul-10	35	4.08	3.59	7.67	13.15
6	20-Jul-10	35	4.06	3.59	7.65	13.11
average		36.17	4.12	3.86	7.98	13.23
target		36	4.00	4.00	8.00	13.30
deviation		0.46%	2.96%	-3.54%	-0.29%	-0.50%

spray pressure:	4 bar
spray volume:	200 L/ha
nozzle type:	Albuz AVI ISO 110 02
spray boom length:	8 m
total number of nozzles:	16

PREPARATION OF TEST SOLUTIONS

treatment	plots treated	water volume (L)	test item quantity (g) (RI: L)	day solution prepared and applied	time solution prepared
Water	3, 8, 21, 27	50	-	20-Jul-10	n.r.
21 g a.s./ha	4, 12, 17, 19	50	30.695	20-Jul-10	13:40
5.1 g a.s./ha	7, 13, 14, 20	50	7.459	20-Jul-10	12:40
1.7 g a.s./ha	5, 10, 15, 22	50	2.488	20-Jul-10	12:10
0.51 g a.s./ha	2, 11, 23, 25	50	0.752	20-Jul-10	11:37
RI	1, 9, 18, 26	50	0.100	20-Jul-10	14:05

a.s. = BYI 02960
a.s. = BYI 02960
a.s. = BYI 02960
a.s. = BYI 02960
RI = Reference Item = lambda-cyhalothrin

Note 1: Water volumes were measured out with a calibrated balance.
Note 2: n.r. means not recorded

APPLIED VOLUMES AND RATES

treatment	plots treated	plot surface (m2)	volume left side (L)	volume right side (L)	total volume applied (L)	actual application volume (L/ha)	standard deviation (L)	deviation from target volume	actual application rate (g a.s./ha)	standard deviation (g)	deviation from target rate (%)
Water	3	484	4.67	5.37	10.04	207		3.72%			
Water	8	484	4.81	5.50	10.31	213		6.51%			
Water	21	484	4.87	5.71	10.58	219		9.30%			
Water	27	484	4.96	5.74	10.69	221		10.43%			
					average	**215**	**6**	**7.49%**			
21 g a.s./ha	4	484	5.28	4.03	9.31	192		-3.82%	20.1929		-3.84%
21 g a.s./ha	12	484	5.35	3.93	9.28	192		-4.13%	20.1278		-4.15%
21 g a.s./ha	17	484	4.67	4.64	9.31	192		-3.82%	20.1929		-3.84%
21 g a.s./ha	19	484	5.37	3.63	9.00	186		-7.02%	19.5205		-7.05%
					average	**191**	**3**	**-4.70%**	**20.0085**	**0.3268**	**-4.72%**
5.1 g a.s./ha	7	484	5.10	3.68	8.78	181		-9.30%	4.6276		-9.26%
5.1 g a.s./ha	13	484	5.53	3.98	9.51	196		-1.76%	5.0124		-1.72%
5.1 g a.s./ha	14	484	5.40	4.15	9.55	197		-1.34%	5.0334		-1.31%
5.1 g a.s./ha	20	484	4.96	3.82	8.78	181		-9.30%	4.6276		-9.26%
					average	**189**	**9**	**-5.42%**	**4.8252**	**0.2284**	**-5.39%**
1.7 g a.s./ha	5	484	6.83	3.28	10.11	209		4.44%	1.7774		4.55%
1.7 g a.s./ha	10	484	4.19	5.00	9.19	190		-5.06%	1.6156		-4.96%
1.7 g a.s./ha	15	484	5.04	4.06	9.10	188		-5.99%	1.5998		-5.89%
1.7 g a.s./ha	22	484	n.r.	n.r.	n.r.	-		-	-		-
					average	**196**	**12**	**-2.20%**	**1.6643**	**0.0983**	**-2.10%**
0.51 g a.s./ha	2	484	4.63	5.36	9.99	206		3.20%	0.5308		4.09%
0.51 g a.s./ha	11	484	4.02	5.27	9.29	192		-4.03%	0.4936		-3.21%
0.51 g a.s./ha	23	484	4.39	5.16	9.55	197		-1.34%	0.5075		-0.50%
0.51 g a.s./ha	25	484	4.24	5.19	9.43	195		-2.58%	0.5011		-1.75%
					average	**198**	**6**	**-1.19%**	**0.5083**	**0.0161**	**-0.34%**
RI	1	484	5.00	4.18	9.18	190		-5.17%	37.9339		-5.17%
RI	9	484	4.72	5.12	9.84	203		1.65%	40.6612		1.65%
RI	18	484	4.91	5.02	9.93	205		2.58%	41.0331		2.58%
RI	26	484	5.58	3.73	9.31	192		-3.82%	38.4711		-3.82%
					average	**198**	**8**	**-1.19%**	**39.5248**	**1.5500**	**-1.19%**

Note 1: n.r. means not recorded

C. Biological Findings

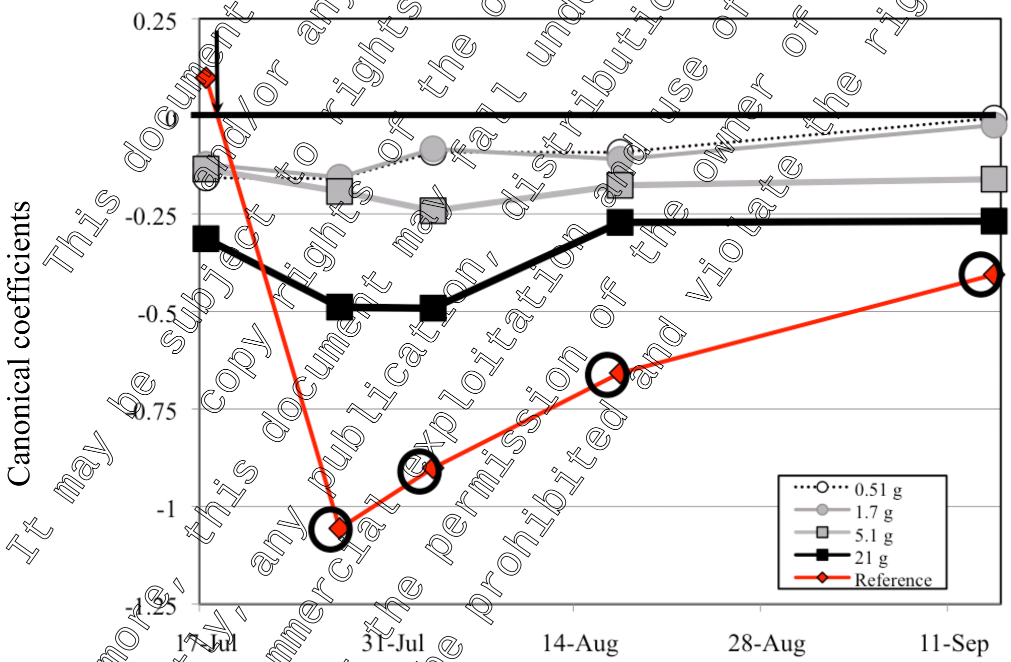
Community level effects

Treatment with the insecticide BYI 02960 SL 200 in an off-field grassland habitat did not lead to statistically significant effects on prevailing arthropod communities at any of the rates tested up to 21 g a.i./ha. Visual inspection of the PRC graph (see figure below) showed differences between the highest two test item rates and the control which were of small magnitude and short duration, which were not statistically significant at any sampling moment.

When examining community responses obtained from separate datasets, it appeared that the mite community (weed samples) and the arthropod community sampled from the soil surface (pitfall samples) did not show adverse responses to any of the BYI 02960 SL 200 treatments. It was only the arthropod community collected with suction sampling that showed signs of transient adverse treatment related effects, but no statistically significant effects were found on any sampling moment in any of the datasets analyzed.

Figure **Principle Response Curve (first ordination axis entire dataset)**

Test- and reference items were analyzed separately but for comparison plotted in one graph. PRC analyses comprised data from weed (W)-, pitfall (P)- and suction (S) samples. Encircled data points are statistically significantly different from the control (Monte-Carlo Permutation test, alpha = 0.05). The arrow indicates the application day.



Population level effects

Three taxa showed statistically significant adverse response patterns that were considered related to the test item treatment. These concerned phytophagous species:

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1) Aphidoidea collected with suction sampling were reduced compared to the control on three consecutive sampling moments after application in the highest BYI 02960 SL 200 rate (21 g a.i./ha). The effect was statistically significant at an alpha level of 0.05 only on the sampling moment one month after application. On the last sampling moment (two months after applications) densities were similar to control densities.

The life cycle of aphids is extraordinary for insects as it includes parthenogenetic generations throughout the season and can be highly dynamic. The number of individuals can drop from one day to another if relative humidity falls below a species-specific threshold. However, quick recoveries from such downfalls are very typical as well and can be attributed to their asexual reproductive potentials. In terms of generations per year, aphids generally produce several (asexual) generations throughout the season. As the Cicadellidae, they are also homopteran phloem sucking insects with mouth parts penetrating plant material

2) Juvenile Cicadellidae collected with suction sampling were statistically significantly reduced compared to the control one and two weeks after application in the highest BYI 02960 SL 200 rate (21 g a.i./ha). On the last two sampling moments (one and two months after application) densities were still reduced compared to the control but differences were no longer statistically significant. Densities in the 5.1 g a.i./ha rate were statistically significantly reduced by 70% on the sampling moment immediately after treatment. During the remainder of the sampling period densities were similar to the control in this test item treatment. These effects on juvenile Cicadellidae were not observed in pitfall sample data.

Cicadellid juveniles and adults are phloem sucking insects with plant-tissue penetrating mouth parts as all other Homoptera. The biology and ecology do not change very much throughout development in Cicadellidae because they are an insect group without a complete metamorphosis (= Hemimetabola). Species in arable habitats on perennial host plants as in grasslands mostly have two or more generations per year with a strong potential for recovery from short-term effects. It can be concluded that the adults at later sampling events are the grown-up juveniles of the earlier sampling events and logically would also occur in a lower abundance. Nevertheless, no response was observed in adult Cicadellids two weeks and six weeks later, respectively. There were even higher numbers of adults relative to the control not only in test plots of 21 g a.i./ha but also in all other plots except for the toxic reference. This supports the assumption that the observed effect at the test rate of 21 g a.i./ha was only of short term. The winged adults are more mobile than the larvae and could quickly stabilize the population by migration and reproduction.

3) Plant eating *Alticinae* (Chrysomelidae, Coleoptera, suction sampling) were statistically significantly reduced in the 5.1 g and 21 g a.i./ha rate one or two weeks after application. Reductions in these two test item treatments varied between approximately 70% and 90%. Differences compared to the control were not statistically significant on the last two sampling moments.

Alticinae are small to moderately sized Chrysomelidae. They are similar to other leaf beetles, but characteristically have the femora greatly enlarged, allowing for springing action when disturbed. Flea beetles can also walk normally and fly, indicating a large potential for recovery. Adult flea beetles feed externally on plants, eating the surface of the leaves, stems and petals.

For few other taxa reductions compared to the control occurred incidentally, but no consistent trend in time or relation to the dose rate was found:

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Juveniles of the plant inhabiting crab spider *Runcinia* (Thomisidae) were moderately but statistically significantly reduced one week after application, both in the 21 g a.i./ha rate and in the 5.1 g a.i./g ha rate. Pre-application densities were however very low. Another taxon of the family Thomisidae, *Xysticus*, was present at higher numbers during application, and showed no adverse response to the test item treatment.

Several microhymenoptera in the two highest test rates (5.1 and 21 g a.i./ha) were moderately reduced compared to the control immediately after application (e.g. several chalcids: Eulophidae (but not the highest rate). However, differences were either not statistically significant, or magnitude of effects were not according to dose rate.

Phoridae (Diptera) collected with suction sampling were statistically significantly reduced one week after application (5.1 g a.i./ha) or two weeks after application (21 g a.i./ha). Otherwise dynamics were similar to the control. Phoridae populations collected with pitfalls were not reduced compared to the control, nor were Phoridae populations in reference treatment plots (suction and pitfall samples).

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Summary table population level effects

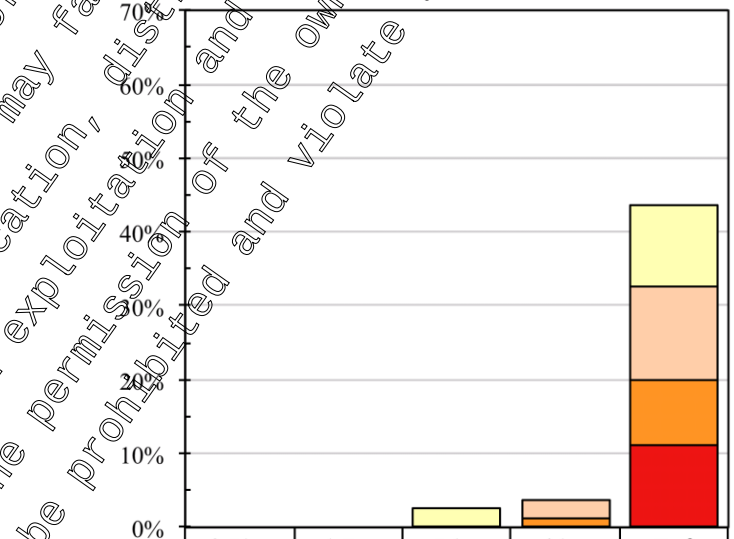
Effect classification:		
one occasion	Clear adverse treatment related effect but observed only on one occasion	2
< 2 months (a)	no longer statistically significant on the last two sampling moments	3a
< 2 months (b)	no longer statistically significant on the the last sampling moment	3b
> 2 months	No recovery within the study period (= 2 months)	8
	Based on 10% significance level and visual consideration of trends	2
	Based on 5% significance level and visual consideration of trends	2

Order	Taxon	method	0.51 g	1.7 g	5.1 g	21 g	Ref
ACARI	Phytoseiidae (female)	W					2
	Total other Gamasida	W					2
	Tetranychidae	W					2
	Oribatida	W					2
ORTHOPTERA	Orthoptera	P					8
HOMOPTERA	Cicadellidae (juveniles)	P					8
HYMENOPTERA	Proctotrupoidea (6)	P					3a
COLEOPTERA	Coleoptera (juveniles)	P					3a
ARANEAE	Thomisoidea	P					8
	Pardosa (juveniles)	P					8
	Pardosa (adult)	P					3a
	Erigoninae	P					8
THYSANOPTERA	Thysanoptera	S					3a
HOMOPTERA	Aphidoidea	S				3b	3b
	Cicadellidae (juveniles)	S			2	3a	8
	Cicadellidae (adults)	S					3a
	Fulgoromorpha	S					3a
DIPTERA	Cecidomyiidae	S					8
	Chloropidae	S					2
	Diptera juveniles	S					8
HYMENOPTERA	Formicidae	S					2
	Trichogrammatidae	S					2
COLEOPTERA	Altiinae	S			2	3a	2
ARANEAE	Phlodromidae juveniles	S					2
	Erigoninae	S					8

Total number of taxa evaluated: 80

W=weed samples/Berlese extraction
P=pitfall
S-suction

According to De Jong *et al.* (2010), recovery should be demonstrated on two consecutive sampling moments. Due to the 4 week interval between the last two sampling moments this would result in a very conservative effect classification. Effect class 3 was therefore subdivided in class 3a (no longer statistically significant on the last two sampling moments) and class 3b (no longer statistically significant on the last sampling moment).



	0.51 g	1.7 g	5.1 g	21 g	Ref
one occasion	0.0%	0.0%	2.5%	0.0%	11.3%
< 2 months (a)	0.0%	0.0%	0.0%	2.5%	12.5%
< 2 months (b)	0.0%	0.0%	0.0%	1.3%	8.8%
> 2 months	0.0%	0.0%	0.0%	0.0%	11.3%

Only taxa that were adversely affected by the test item or by the reference item are shown.

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Summary effect classifications

Effects of BYI 02960 SL 200 applied to an off-crop grassland arthropod fauna in South-West France are classified as follows:

Table 10.5.4- 6: Effect classifications of BYI 02960 SL 200 rates applied in an off-crop grassland in The Netherlands

Community level effects (PRC/Monte-Carlo; 5% alpha level)	**0.51 g a.s./ha**	**1.7 g a.s./ha**	**5.1 g a.s./ha**	**21 g a.s./ha**
	Effect class			
Conclusion	1	1	1	1 **Community NOER**
Population level effects (Mann-Whitney U test; 5% alpha level)	**0.51 g a.s./ha**	**1.7 g a.s./ha**	**5.1 g a.s./ha**	**21 g a.s./ha**
	Effect class			
Aphidoidea (Homoptera)	1	1	1	3b
Juvenile Cicadellidae (Homoptera)	1	1	2	3a
Alticinae (Chrysomelidae, Coleoptera)	1	2	2	3a
Conclusion		**Population NOER**		**Population NOEAER**

NOER: No Observed Effect Rate (no statistically significant differences compared to control)

NOEAER: No Observed Ecologically Adverse Effect Rate (at least 1 taxon with effect class 2 or 3, i.e. clear response to treatment with recovery within 2 months after application)

D. Biological Endpoints Derived

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

NOER (community)	21 g a.i./ha in an off-crop grassland
NOER (population)	1.7 g a.i./ha in an off-crop grassland
NOEAER (population)	21 g a.i./ha in an off-crop grassland

CONCLUSION

The community NOER (No Observed Effect Rate) of BYI 02960 SL 200 applied in an off-crop grassland in South-West France is 21 g a.i./ha.

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Only three taxa were adversely affected. At the test rate of 5.1 g a.i./ha these effects were only on single sampling occasions significant and at the rate of 21 g a.i./ha they all recovered within the ecologically acceptable time frame of two months. The rate of 1.7 g a.i./ha is classified as the population NOER (no observed effect rate) and the highest rate tested in this study, BYI 02960 SL 200 applied at 21 g a.i./ha, is therefore classified as the population NOEAER (no observed ecologically adverse effect rate).

IIIA1 10.6 Effects on earthworms and other soil macro-organisms

The risk assessment procedure follows current regulatory requirements and the Guidance Document on Terrestrial Ecotoxicology. The summary of the toxicity of BYI 02960 SL 200, BYI 02960 and the relevant metabolites to earthworms is provided in the tables below. Details of the studies with BYI 02960 and relevant metabolites are provided in the Tier II summary document on the active substance Annex II, Point 8.9 (Earthworms) and Point 8.14 (other soil macro-organisms).

The risk assessment presented below gives clear evidence that earthworms and other soil macro-organisms are not at risk if BYI 02960 SL 200 is applied according to the recommended use pattern. Acute and chronic studies are available with *Eisenia fetida*, and chronic studies with *Folsomia candida* and *Hypoaspis aculeifer*. The collembolan species *F. candida* was determined to be the most sensitive species to BYI 02960 SL 200 in soil with a NOEC (reproduction) of 8.47 mg BYI 02960 SL 200/kg. However, a TER of 12 for *F. candida* indicates that collembolan populations are not at risk if BYI 02960 SL 200 is applied at rates of 0.625 L/ha in Lettuce (this also covers the use in hops with a lower PEC_{soil}). In addition, higher tier studies (earthworm field study and a litterbag study) are available with BYI 02960 SL 200 revealing that natural earthworm populations and the process of organic matter degradation in soil are not at risk if BYI 02960 SL 200 is applied according to the recommended use pattern.

The two metabolites DFA and 6-CNA were investigated for their chronic impact on *E. fetida*, *F. candida* and *H. aculeifer*. The lowest endpoint in laboratory tests with a metabolite was observed in the earthworm reproduction test with DFA (NOEC=62 mg/kg). However, TER values of ≥ 4429 demonstrate an overall low ecotoxicological risk arising from the metabolites in soil and a high margin of safety.

Overall, considering the available data package it can be concluded that earthworms and other soil organisms are not at risk if BYI 02960 SL 200 is applied at rates of 0.75 L/ha in Hops or 0.625 L/ha in Lettuce, respectively.

The summary of the toxicity of BYI 02960 SL 200, BYI 02960 and relevant metabolites to earthworms is provided in the tables below and/or in the Tier II summary document on the active substance Annex II, Point 8.9 (Earthworms) and Point 8.14 (*Folsomia* and *Hypoaspis*).

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Acute and chronic toxicity of BYI 02960 and the metabolites difluoroacetic acid and 6-chloronicotinic acid to earthworms

Table.10.6- 1: Effects of BYI 02960 and relevant metabolites on soil macro-organisms – earthworms

Test species	Test design	Ecotoxicological endpoint	Reference
BYI 02960			
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50} 192.9 mg a.i./kg dws	[redacted] (2010) M-363742-01-1 KIIA 8.9.1/01
DFA			
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50} > 1000 mg p.m./kg dws	[redacted] (2007) M-368835-01-1 KIIA 8.9.1/02
Eisenia fetida	reproduction, 56 d (10% peat in test soil) mixing	NOEC 62.6 mg p.m./kg dws	[redacted] (2010) M-398061-01-1 KIIA 8.9.2/02
6-CNA			
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50} > 1000 mg p.m./kg dws	[redacted] (1999) M-196591-01-1 KIIA 8.9.1/03
Eisenia fetida	reproduction, 56 d (10% peat in test soil) mixing	NOEC 95 mg p.m./kg dws	[redacted] (2011) M-413562-02-1 KIIA 8.9.2/03

dws = dry weight soil
a.i. = active ingredient; p.m. = pure metabolite

Acute and chronic toxicity of BYI 02960 SL 200 G to earthworms

Table.10.6- 2: Effects of BYI 02960 SL 200 G on soil macro-organisms - earthworms

Test species	Test item	Test design	Ecotoxicological endpoint	Reference
Eisenia fetida	BYI 02960 SL 200 G	acute, 14 d (5% peat in test soil)	LC_{50} 709 mg prod./kg dws	[redacted] (2010) M-397720-01-2 KIIIA1 10.6.2/01
Eisenia fetida	BYI 02960 SL 200 G	reproduction, 56 d (10% peat in test soil)	NOEC 8.9 mg prod./kg dws	[redacted] (2010) M-392964-01-2 KIIIA1 10.6.3/01,
Earthworm fauna	BYI 02960 SL 200 G	Field study on grassland one year 300, 600 and 1500 g a.i./ha	Significant reduction of abundance (-33%) and biomass (-38%) at 1500 g a.i./ha and biomass at 600 g a.i./ha (-36%) after 1 month; full recovery of earthworm population after 11 months	[redacted] (2012) M-426607-01-1 KIIIA1 10.6.4/01

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Exposure in soil

Predicted environmental concentrations in soil (PEC_{soil}) values were calculated for the active ingredient and its metabolites as described in detail in Point 9.4 (active substance) and 9.5 (metabolites).

A soil layer of 5 cm with a bulk density of 1.5 g/cm^3, 60 % interception in hops and 25 % interception in lettuce and a worst case DT_{50} of 468 days for BYI 02960 for long-term accumulation was considered. For the formulation, application of 0.75 L/ha in hops and 0.625 L/ha in lettuce with a product density of 1.175 g/cm^3 with the same interception rates as for the active substance was assumed.

The maximum PEC_{soil} values are summarised in the following table.

Table.10.6- 3: Maximum PEC_{soil} values

Crop / appl. rate	Hops 1 x 150 g a.i./ha		Lettuce 1 x 125 g a.i./ha	
Compound	**$PEC_{soil, max}$ [mg/kg]**	**$PEC_{soil accu}$ [mg/kg]**	**$PEC_{soil, max}$ [mg/kg]**	**$PEC_{soil accu}$ [mg/kg]**
BYI 02960 SL 200	0.470	-	0.685	-
BYI 02960	0.080	0.160	0.125	0.156
Difluoroacetic acid	0.009	-	0.014	-
6-chloronicotinic acid	0.007	-	0.012	-

IIIA1 10.6.1 Toxicity exposure ratios for earthworms, TER_A and TER_{LT}

Based on most sensitive endpoints the TER values are calculated using the following equations:

$TER_A = LC_{50} / PEC_{soil}$

TER_{LT} = chronic NOEC / PEC_{soil}

The risk is considered acceptable, if the TER_A is >10 and the TER_{LT} is >5.

For lipophilic substances (log P_{OW} > 2) the Terrestrial Guidance Document recommends to apply an additional assessment factor of 2 for the ecotoxicological endpoints (LC_{50}, NOEC), if the study was conducted in artificial soil with a high content of organic matter (i.e. 10 % peat), to consider the possible sorption of these compounds to the organic matter. However, BYI 02960 and its metabolites have a log P_{OW} < 2 and no additional assessment factor has to be applied.

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Table 10.6.1- 1: TER calculations for earthworms

Compound test design	End point	[mg/kg soil]	$PEC_{max,accu}$ [mg/kg soil]	TER_A / TER_{LT}	Trigger	Refined risk assessment?
Hops						
BYI 02960 SL 200 acute	LC_{50}	709	0.470	1509	10	
BYI 02960 SL 200 chronic	NOEC	8.9	0.470	19	5	
BYI 02960 acute	LC_{50}	192.9	0.160	1206	10	
DFA acute	LC_{50}	> 1000	0.009	> 111 111	10	No
DFA chronic	NOEC	62.0	0.009	6889	5	
6-CNA acute	LC_{50}	≥ 1000	0.007	≥ 142 857	10	
6-CNA acute	NOEC	95	0.007	13 571	5	
Lettuce						
BYI 02960 SL 200 acute	LC50	709	0.685	1035	10	
BYI 02960 SL 200 chronic	NOEC	8.9	0.685	13	5	
BYI 02960 acute	LC50	192.9	0.156	1237	10	
DFA acute	LC50	> 1000	0.014	> 71 429	10	No
DFA chronic	NOEC	62.0	0.014	4429	5	
6-CNA acute	LC50	≥ 1000	0.012	≥ 83 333	10	
6-CNA acute	NOEC	95	0.012	7917	5	

Conclusion: The TER values are above the trigger of concern, indicating no unacceptable risk for earthworms and soil non-target macro-organisms.

IIIA1 10.6.2 Acute toxicity to earthworms

Report:	**KIIIA1 10.6.2/01; [redacted], 2010**
Title:	BYI 02960 SL200 G: acute toxicity to earthworms (*Eisenia fetida*) tested in artificial soil with 5% peat
Report No:	LRT/Rg-A-143/10
Document No:	M-397720-01-2
Guidelines:	**OECD-Guideline No. 207 (1984)**
Deviations:	**None**
GLP	**Yes (certified laboratory)**

Executive Summary

The aim of the study was to determine the acute effects of BYI 02960 SL 200 G (Specification No. 102000021884-01; Sample description: TOX 08907-00; Batch ID: 2010-001067; Material No.: 79718845; density 1.175 g/mL, content 201.0 g BYI 02960/L = 17.1% w/w) to earthworms (*Eisenia fetida andrei*).

Adult earthworms (more than two months old) were exposed in an artificial soil system with a peat content of 5% over a period of 14 days to nominal concentrations of 100, 178, 316, 562, 1000 and 1780 mg product/kg dry weight soil, respectively. In addition, a water control was tested as negative control. Mortality and sublethal behavioural effects were determined.

The 14-day-LC_{50} was 709 mg product/kg dry weight soil (95% confidence limits: 245 to 2187 mg/kg), the 14-day-NOEC was determined to be < 100 mg product/kg dry weight soil.

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MATERIAL AND METHODS

A. Materials

1. Test material

Test item:	BYI 02960 SL 200 G
Type:	Formulated product
Specification No.:	102000021884-01
Material number:	79718845
Sample description:	TOX08907-00
Batch No.:	2010-001067
Nominal content of active substance:	BYI 02960: 200 g/L
Analytical content of active substance:	BYI 02960: 17.1 % w/w, 201.0 g/L according to certificate of analysis
Density:	1.175 g/mL at 20°C
Stability of test item:	Expiry date: 2012-06-14, when stored at 25±5 °C

2. Vehicle and/or positive control:

Test item mixed with:	Deionized water
Controls:	Water control

3. Test organisms

Species:	*Eisenia fetida andrei*
Common name:	Earthworm
Source:	In-house lab culture
Age at study initiation:	More than two months old
Feeding during test:	None
Weight at test start:	0.31 g
Maintenance of culture:	
Temperature:	22 ± 2°C
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 15 to 30, 2010

2. Design of biological test

Adult earthworms (*Eisenia fetida andrei*; more than two months old) were exposed to BYI 02960 SL 200 G; (purity 201.0 g BYI 02960/L = 17.1% w/w) in an artificial soil system with 5 % peat over a period of 14 days. Nominal concentrations were 100, 178, 316, 562, 1000 and 1780 mg test item/kg dry weight soil. In addition a water control was tested. Each jar (glass jar; 1.5 L) served as one replicate filled with 595 g dry weight test soil (equivalent to 750 g wet 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 at constant light. The artificial soil contained 5% peat, 20% kaolinite clay, 74.8% quartz sand and 0.2% calcium carbonate. Incubation conditions during the study were constant light (400 - 800 Lux, integrated luxmeter of the climatic chamber) and a temperature of 20 ± 2 °C.

3. Observation and measurements

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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.

4. Statistical analysis

The LC_{50}-values and the 95 percent confidence limits were calculated by Probit-Analysis according to "Maximum-Likelihood" Method ([redacted]. 1978). The data on weight alteration of the test organisms after 2 weeks of exposure were statistically analysed with Williams multiple t-test (alpha = 0.05, one-sided smaller). Previous statistical testing of the data showed that they fitted the assumption of normal distribution (Kolmogorov-Smirnov test) and showed homogeneity of variances as checked by Cochran´s test. The statistic software used was ToxRatPro Version 2.09 @.

RESULTS AND DISCUSSION

A. Physical and Chemical Parameters

The soil-pH was 6.12 to 6.20. The water content was 57.1 to 57.8% of the maximum water holding capacity.

B. Biological Findings

No morphological and behavioural effects were observed. Acute toxicity of the formulation to earthworms after 14 days is as follows:

Table 10.6.2- 1: Effects of BYI 02960 SL 200 G on mortality and body weight chance of *Eisenia fetida andrei*

Nominal Concentration of test item (mg/kg dry soil) [a]	% mortality [b] after 7 days	% mortality [b] after 14 days	% weight alteration of the survivors [b]	significance from control Williams-test [c]
control	0	0	- 4 ± 4	
100	0	0	- 16 ± 6	+
178	0	3 ± 6 [d]	- 22 ± 5	+
316	0	0	- 28 ± 3	+
562	3 ± 5 [b]	28 ± 15	- 36 ± 5	+
1000	50 ± 14	78 ± 21	- 48 ± 1	+
1780	98 ± 5	100	---	---

+ = weights of control and the test concentration do differ statistically significantly
= weights of control and the test concentration do not differ statistically significantly
(a) test concentrations are nominal concentrations
(b) mean ± standard deviation
(c) Williams-Test (alpha = 0.05, one-sided smaller)
(d) refers to one dead worm in one replicate

C. Validity Criteria

There was no mortality observed in the control vessels. 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:	DEC 2009
Result:	14 day LC_{50}: 26.1 mg Chloroacetamide A.R./kg dry weight soil

E. Biological Endpoints Derived

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

14-day-figures	
highest concentration with no effect (NOEC):	100 mg test item/kg dry weight soil
lowest concentration with effect (LOEC):	100 mg test item/kg dry weight soil
LC50:	709 mg test item/kg dry weight soil (95% confidence limits: 245 to 2187 mg/kg)

CONCLUSION

The acute effect of BYI 02960 SL 200 G on earthworms (*Eisenia fetida andrei*) can be quantified as a 14-day-LC_{50} of 709 mg test item/kg dry weight soil (95% confidence limits: 245 to 2187 mg/kg). The highest concentration with no mortality and no sublethal behavioural effects can be set to < 100 mg test item/kg dry weight soil.

IIIA1 10.6.3 Sublethal effects on earthworms

Report:	**KIIIA1 10.6.3/01; [redacted] T., 2010**
Title:	BYI 02960 SL 200 G: Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil
Report No:	LRT-RG-R-76/09
Document No:	M-392964-01-2
Guidelines:	**ISO 11268-2, 1998 (E) and OECD 222 (2004)**
Deviations:	**None**
GLP:	**Yes (certified laboratory)**

The full summary of this study is reported in the Annex II document, as it is a core requirement (see KIIA 8.9.2/01). However, a short overview is presented below.

Executive Summary

The aim of the study was to determine the effects of BYI 02960 SL 200 G (Specification No. 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 month old) were exposed in an artificial soil system over a period of 56 days to nominal concentrations of 8.9, 15.8, 28.1, 50.0 and 89.0 mg product/kg dry weight soil, 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.

The overall NOEC was determined to be 8.9 mg product/kg dry weight soil, based on reproduction.

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IIIA1 10.6.4 Field tests (effects on earthworms)

Report:	**KIIIA1 10.6.4/01; [redacted], 2012**
Title:	BYI 02960 SL 200 G: Effect on the earthworm fauna of a grassland area with one year
Report No:	MNU/Rg-F-8/12
Document No:	M-426607-01-1
Guidelines:	**ISO CD 11268-3 (E), 1999, BBA Part VI, 2-3, 1994, OPPTS 850.supp**
Deviations:	**None**
GLP:	**Yes (certified laboratory)**

Objective

This test was designed to determine the effects of BYI 02960 SL 200 G (a.i. 200 g/L) on earthworm populations under field conditions.

Material and methods

The effects of BYI 02960 SL 200 G (analysed content: 201 g/L, Density: 1.175 g/mL, Batch-ID: 210-001067, TOX-No.: 08907-00) on earthworm populations under field conditions were studied. To ensure an abundant earthworm population, an area was selected which was used as grassland for four years, located on Bayer Experimental Farm [redacted], Germany. On May 17, 2010 a pre-sampling of earthworms was conducted to ensure a sufficient abundance of earthworms being present at the test plot.

On May 25, 2010, BYI 02960 SL 200 G was applied at rates of 300, 600 and 1500 g a.i./ha per four treatment plots (10 x 10 m) on a test area of 60 x 20-40 m in size. Four untreated plots served as controls. Four plots were used as positive controls and were treated with Carbendazim with an application rate of 10 kg a.i./ha. Within nine days after application 59.4 mm of precipitation was measured. All plots were screened for alive and dead earthworms on the soil surface one, two and six days after the applications.

The earthworm abundance and biomass was sampled four weeks (June 22 - 23, 2010), five months (October 27 - 28 and November 5, 2010) and eleven months after the application (April 5 - 6, 2011), respectively by sampling using formalin method. At each sampling time point 16 samples per treatment (4 plots, 4 samples per plot) were collected.

Soil samples from the control and from the treated plots were taken on May 26, 2010. Soil samples were analysed according to method 01074/M001 (described in the conjunct study MR-11/27) for the presence of BYI 02960.

Findings

The present earthworm field study shows that BYI 02960 SL 200 G applied with application rates of 300, 600, 1500 g a.i./ha, has no unacceptable adverse effect on the population of earthworms five and eleven months after the application date (Table). Compared to control plots, plots treated with BYI 02960 SL 200 G showed insignificant changes of the relative abundance of adult & juvenile earthworms between +7 and -13 % (abundance) and -7 and -10 % (biomass) five months after application of BYI 02960 SL 200 G. Eleven months after application a relative increase between +1 and 14 % (abundance) and +1 and +10 % (biomass) was observed (all not significant).

Four weeks after application of BYI 02960 SL 200 G a significant reduction of adult & juvenile earthworms of -33 % (abundance) at the application rate of 1500 g a.i./ha was observed. At an application rate of 600 g a.i./ha, a reduction in the total biomass of adult and juvenile earthworm by -33 % (significant) and no significant change in the abundance compared to control plots was observed.

With respect to the diversity indices of SHANNON-WEAVER the earthworm community on the test site is comparable to normal findings in our latitudes (BAUCHHENSS 1982). Overall, the diversity index for BYI 02960 SL 200 G is in the same range for any reading point as in the control plots for adult and juvenile earthworms also indicating that BYI 02960 SL 200 G does not adversely affect the earthworm population.

The treatment with the reference substance Carbendazim showed strong effects four weeks and five months after the application on the earthworm community in comparison to the control. The reference substance applied at a rate of 10 kg a.i./ha did decrease the abundance of earthworms by -97 % (four weeks after application). Therefore, the reference item treatment confirmed the sensitivity of the earthworm population under the specific experimental conditions and the validity of the study, as recommended by Kula *et al*. (2006).

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Table 10.6.4- 1: Changes in abundance and biomass for juvenile & adult earthworms, summary

The values are replicate means (n = 4) ± standard deviations per 0.25 m². Values between parentheses are relative differences to the control in %:

Treatment group (g a.i./ha)	4 weeks after the application		5 months after the application		11 months after the application	
	Relative abundance of juvenile & adult earthworms in the study plots (from replicate means)					
	Total earthworms					
Control	17.00 ± 2.68		100.44 ± 13.22		66.06 ± 15.89	
BYI 300	19.81 ± 8.00	(+17 %)	92.75 ± 27.75	(- 8 %)	66.44 ±24.06	(+ 1 %)
BYI 600	13.38 ± 5.46	(- 21 %)	107.56 ± 18.79	(+ 7 %)	75.19 ±11.79	(+14 %)
BYI 1500	11.44 ± 3.72	(- 33 %) *	86.94 ± 30.46	(-13 %)	68.88 ±10.42	(+ 4 %)
Carbendazim	0.50 ± 1.00	(- 97 %) *	52.44 ± 22.83	(-48 %) *	52.50 ± 16.41	(- 21 %)
	Relative changes of biomass [g] of juvenile & adult earthworms in the study plots (from replicate means)					
Control	10.22 ± 2.16		45.71 ± 4.35		34.78 ± 5.31	
BYI 300	12.19 ± 5.62	(+19%)	42.61 ± 5.58	(- 7 %)	35.25 ±2.21	(+ 1 %)
BYI 600	6.87 ± 1.29	(-33%) *	40.98 ± 3.92	(-10 %)	38.21 ±6.38	(+10 %)
BYI 1500	6.61 ± 2.26	(-35%)	42.20 ± 11.41	(- 8 %)	36.30 ±4.86	(+ 4 %)
Carbendazim	0.33 ± 0.66	(-97%) *	22.00 ± 8.71	(-52 %) *	31.21 ± 7.96	(- 10 %)

***)** Significant difference from control according to the U-test, two sided at the significance level alpha = 0.05 (U-test from Wilcoxon, Mann and Whitney after SACHS 1978).

Total earthworm population; changes of abundance & biomass

BYI 02960 SL 200 G applied with application rates of 300, 600, 1500 g a.i./ha has no statistically significant effect on the parameters “abundance” and “biomass” of the category total earthworms five months and 11 months after the application. This indicates that BYI 02960 at the rate tested has no effect on the earthworm community. Five months after application the total abundance of earthworms was between +7 and -13 % and the total biomass of earthworms between -7 and -10 % (biomass) at all application rates (all not significant). Eleven months after application the abundance of total earthworms increased between 1 and 14 % compared to control and the biomass of total earthworms increased between 1 and 10 % (all not significant). This variation is considered to be in the range of natural variability of the earthworm population. Four weeks after application of BYI 02960 SL 200 G showed a significant reduction of adult & juvenile earthworms of -33% (abundance) at an application rate of 1500 g a.i./ha. At an application rate of 600 g a.i./ha, a significant reduction in the total biomass of adult and juvenile earthworm by -33 % and no significant change in the abundance compared to control plots.

The data were further analysed with respect to the different ecological groups of earthworms. For anecic earthworms no statistically significant difference in abundance and biomass in treated plots compared to control was observed. Only, five months after application at an application rate of 600 g a.i./ha, the abundance of anecic earthworms sampled on treated plots was 57 % (significant) lower than the abundance on control plots and biomass in treated plots was 61 % (significant) lower than on control plots.

Also for the group of endogeic earthworms no statistically significant difference between control and treated plots was observed with respect to abundance and biomass, except five months after application at the application rate of 1500 g a.i./ha. Here, the biomass of total endogeic earthworms sampled on treated plots was -42 % below the biomass found on control plots.

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Four weeks after application at the application rate 1500 g a.i./ha the abundance of epigeic earthworms was significantly reduced by -32 % on treated plots compared to control plots. All other differences between treated plots and control plots were not statistically significant.

Overall it can be concluded that 11 months after application of BYI 02960 no unacceptable adverse effects on adult & juvenile earthworms were observed.

The effects induced by the use of Carbendazim 4 weeks after application with a decrease in the abundance of earthworms by -97 % clearly show the sensitivity of the test.

Conclusion

The present earthworm field study shows, that BYI 02960 SL 200 G applied with application rates of 300, 600 and 1500 g a.i./ha has no unacceptable adverse effect on the population of earthworms five months and 11 months after the application. Only, four weeks after application BYI 02960 SL 200 G affected a significant reduction of adult & juvenile earthworms of -33 % (abundance) at application rate of 1500 g a.i./ha and -33 % (biomass) at application rate of 600 g a.i./ha. However, full recovery of the earthworm population was observed 11 months after the application of BYI 02960 SL 200 G.

IIIA1 10.6.5 Residue content of earthworms

According to current regulatory requirements a log P_{ow} > 3 is used to indicate that there might be a potential for bioaccumulation, as the log P_{ow} of BYI 02960 and the metabolites is < 3 there is no potential for accumulation.

IIIA1 10.6.6 Effects on other soil non-target macro-organisms

Effects of BYI 02960 and its metabolites on other non-target macro-organisms

The summary of the toxicity of BYI 02960 metabolites and BYI 02960 SL 200 to other soil non-target macro-organisms is provided in Table 10.6.6- 1 and Table 10.6.6- 2.

Table 10.6.6- 1: Effects on other soil non-target macro-organisms

Test species	Test design	Ecotoxicological endpoint	Reference
DFA			
Folsomia candida	chronic, 28 d (5% peat in test soil)	NOEC ≥ 100 mg p.m./kg dws	[redacted] (2010) M-368675-01-1 KIIA 8.14/02
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	NOEC ≥ 1000 mg p.m./kg dws	[redacted] (2010) M-390091-01-1 KIIA 8.14/05
6-CNA			
Folsomia candida	chronic, 28 d (5% peat in test soil)	NOEC 90 mg p.m./kg dws	[redacted] (2010) M-407861-01-1 KIIA 8.14/03
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	NOEC ≥ 100 mg p.m./kg dws	[redacted], A. (2011) M-404434-01-1 KIIA 8.14/06

dws = dry weight soil
p.m. = pure metabolite

Effects of BYI 02960 SL 200 G on other non-target macro-organisms

Table 10.6.6- 2: Effects on other soil non-target macro-organisms

Test species	Test design	Ecotoxicological endpoint	Reference
BYI 02960 SL 200 G			
Folsomia candida	chronic, 28 d (5% peat in test soil)	NOEC 8.47 mg prod./kg dws	[redacted] (2009) M-359728-01-2 KIIA 8.14/01, KIIIA1 10.6.6/01
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	NOEC ≥ 1000 mg prod./kg dws	[redacted] (2010) M-358752-01-2 KIIA 8.14/04, KIIIA1 10.6.6/02

dws = dry weight soil
prod. = product

Chronic toxicity exposure ratios for soil non-target macro-organisms

Ecotoxicological endpoints and PEC_{soil} used for TER calculations for soil non-target macro-organisms are summarised below. TER values were calculated using the equation:

$$TER = NOEC / PEC_{soil}$$

The risk is considered acceptable, if the TER_{LT} is >5.

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Table 10.6.6- 3: TER calculations for soil macro-organisms

Compound Test design	Endpoint	[mg/kg soil]	PEC_{max} * [mg/kg soil]	TER	Trigger	Refined risk assessment?
Folsomia candida						
BYI 02960 SL 200 chronic	NOEC	8.47	0.685	12	5	No
6-CNA chronic	NOEC	90	0.012	7500	5	
DFA chronic	NOEC	≥100	0.014	≥ 7143	5	
Hypoaspis aculeifer						
BYI 02960 SL 200 chronic	NOEC	≥ 1000	0.685	≥ 1460	5	No
6-CNA chronic	NOEC	≥100	0.012	≥ 8333	5	
DFA chronic	NOEC	≥1000	0.014	≥71 429	5	

* maximum PEC_{soil} values calculated for the use in lettuce were used for risk assessments, covering the use in hops

Conclusion: The TER values are above the trigger of concern, indicating no unacceptable risk for soil non-target macro-organisms, i.e. collembola, soil mites.

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Effects on other soil non-target macro-organisms

Report:	**KIIIA1 10.6.6/01; [redacted], 2009**
Title:	BYI 02960 SL 200 G: Influence on the Reproduction of the Collembola Species *Folsomia candida* tested in Artificial Soil with 5% Peat
Report No:	FRM-COLL-75/09
Document No:	M-359728-01-2
Guidelines:	**ISO 11267 (1999)**
Deviations:	**To fulfill the recommendations of the proposal for a new OECD guideline 5% peat instead of 10% peat in the artificial soil was tested.**
GLP	**Yes (certified laboratory)**

The full summary of this study is reported in the Annex II document, as it is a core requirement (see KIIA 8.14/01). However, a short overview is presented below.

Executive Summary

The aim of the study was to determine the chronic effects of BYI 02960 SL 200 G (Sample description: FAR01438-00 (Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 102000021884; purity 199.8 g BYI 02960/L; 17.0% w/w) to springtails (*Folsomia candida*).

Ten springtails (10 to 12 days old) per replicate (5 replicates per treatment group) were exposed in an artificial soil system with a peat content of 5 % over a period of 14 days to nominal concentrations of 8.8, 13.2, 19.9, 29.8 and 44.6 mg test item/kg artificial soil dry weight corresponding to 1.5, 2.3, 3.4, 5.1 and 7.6 mg a.i./kg dry weight soil in the 1^{st} 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 a.i./kg dry weight soil in the 2^{nd} run. Since the first test run on BYI 02960 SL 200 G did not provide a final result, a second test run was performed studying lower concentrations. In addition a water control was tested.

Mortality and reproduction were determined after 28 days.

The overall 28-day NOEC was determined to be 8.47 mg product/kg soil dry weight.

Report:	**KIIIA1 10.6.6/02; [redacted] A., 2009**
Title:	BYI 02960 SL 200 G: Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil with 5% peat
Report No:	Kra-HR-19/09
Document No:	M-358752-01-2
Guidelines:	**OECD Guideline No. 226 (2008)**
Deviations:	**To fulfill the recommendations of the proposal for a new OECD guideline 5% peat instead of 10% peat in the artificial soil was tested.**
GLP:	**Yes (certified laboratory)**

The full summary of this study is reported in the Annex II document, as it is a core requirement (see KIIA 8.14/04). However, a short overview is presented below.

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Executive Summary

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) to predatory soil mites (*Hypoaspis 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 nominal 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 1000 mg product/kg dry weight soil.

IIIA1 10.6.7 Effects on organic matter breakdown

According to the current regulatory requirements and the Guidance Document on Terrestrial Ecotoxicology, a study on organic matter breakdown is required based on the $DT90_f$ value of the active substance. A litterbag study is available with BYI 02960 SL 200 G. This study is summarized in detail in the Annex II dossier (KIIA 8.16.2/01) as it represents a core data requirement in the Annex II dossier for the active substance. However, a summary table is presented below.

Table 10.6.7- 6: Effects on organic matter breakdown of BYI 02960

Test species	Test design	Ecotoxicological endpoint		Reference
BYI 02960 SL 200 G				
Soil litter degradation (spraying)	217 d	no influence	soil treated with 150 g a.i./ha for plateau concentration + the annual rate of 300 g a.i./ha)	degradation: 0-29 d: control 23.37% treated 21.56% 0-92 d: control 52.95% treated 62.73% 0-217 d: control 76.38% treated 74.06%	[redacted] (2011) M-413408-01-1 KIIA 8.16.2/01
Soil litter degradation (seed treatment)	217 d	no influence	soil treated with 150 g a.i./ha for plateau concentration + the annual rate in form of treated summer wheat seed (265 g a.i./ha)	degradation: 0-29 d: control 23.37% treated 23.37% 0-92 d: control 52.95% treated 52.35% 0-217 d: control 76.38% treated 80.08%	[redacted] (2011) M-413416-01-1 KIIA 8.16.2/02

IIIA1 10.7 Effects on soil microbial activity

Laboratory studies on microbial turnover are available for the active substance BYI 02960, the formulation BYI 02960 SL 200 G, and the metabolite 6-CNA (N-turnover only).

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The results indicate that BYI 02960 and BYI 02960 SL 200 G have no adverse impact on microbial C- and N-turnover in soil. NOECs were clearly above the expected exposure in soil.

The metabolite 6-CNA was shown to be of general low toxicity for soil organisms (see Table 10.6.1-2). The NOEC of the metabolite 6-CNA for microbial N-turnover was determined to be 111- fold higher than the worst case PEC in soil for 6-CNA. The high margin of safety indicates that microbial C-turnover is not at risk due to formation of 6-chloronicotinic acid in soil. Similar to 6-chloronicotinic acid the metabolite DFA acid was shown to be of general low toxicity for soil organisms (see Table 10.6.1- 3). Assuming a 10-fold higher toxicity of DFA in comparison with to 6-CNA with regard to microbial C- and N-turnover, the risk could still be considered as acceptable.

Overall, it can be concluded that the functioning of soil micro-organisms is not at risk if BYI 02960 SL 200 G is applied according to the recommended use pattern.

The toxicity of BYI 02960 SL 200 G on soil non-target micro-organisms is summarised in Table 10.7-1.

Table 10.7- 1: Effects on soil non-target micro-organisms

Test species	Test design	Ecotoxicological endpoint		Reference
BYI 02960 SL 200 G				
C-cycle	28 d	no influence	1.244 L prod./ha (≡1.66 µL prod/kg dws) 12.44 L prod./ha (≡16.59 µL prod./kg dws)	[redacted] (2010) M-395469-01-2 KIIIA1 10.7.1/01
N-cycle	28 d	no influence	1.244 L prod./ha (≡1.66 µL prod/kg dws) 12.44 L prod./ha (≡16.59 µL prod./kg dws)	[redacted] (2010) M-396112-01-2 KIIIA1 10.7.1/02
BYI 02960				
N-cycle	28 d	no influence	0.3 kg a.i./ha (≡0.4 mg a.i./kg dws) 3 kg a.i./ha (≡4.0 mg a.i./kg dws)	[redacted] (2009) M-359803-01-1 KIIA 8.10.1/01
C-cycle	28 d	no influence	0.3 kg a.i./ha (≡0.4 mg a.i./kg dws) 3 kg a.i./ha (≡4.0 mg a.i./kg dws)	[redacted] (2011) M-417194-01-1 KIIA 8.10.2/01
6-CNA				
N-cycle	28 d	no influence	1.0 kg p.m./ha (≡1.33 mg p.m./kg dws)	[redacted] (2011) M-408028-01-1 KIIA 8.10.1/02

Risk assessment

According to current regulatory requirements the risk is acceptable, if the effect of the recommended application rate of a compound / product on nitrogen or carbon mineralisation is < 25% after 100 days.

Deviations from the control did not exceed 25% after 28 days, indicating low risk to soil micro-organisms.

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IIIA1 10.7.1 Laboratory test to investigate impact on soil microbial activity

Report:	**KIIIA1 10.7.1/01; [redacted] (2010)**
Title:	BYI 02960 SL 200 G: Determination of effects on carbon transformation in soil
Report No:	EBRVP084
Document No:	M-395469-01-2
Guidelines:	**OECD guideline 217, 2000**
Deviations:	**None**
GLP:	**Yes (certified laboratory)**

Executive Summary

BYI 02960 SL 200 G (analytical finding: 201.0 g/L, specification No.: 102000021884-01, batch No.: 2010-001067, master recipe ID: 0102142-001, sample description: TOX 08907-00, density: 1.175 g/mL) was used in the test. The objective of the test was to determine the influence of 1.66 µL and 16.59 µL test item/kg dry weight soil on carbon transformation (glucose-stimulated respiration) in an agricultural soil.

A loamy sand soil was exposed for 28 days to 1.66 µL and 16.59 µL test item/kg dry weight soil, respectively (application rates were equivalent to 1.244 L and 12.44 L test item/ha, respectively). 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.

The deviation from the control did not exceed 25% after 28 days. When used at rates up to 12.449 L test item/ha, BYI 02960 SL 200 G should not have an impact on carbon transformation in soils.

Material and Methods

A. Materials

1. Test material

Test item:	BYI 02960 SL 200
Specification No.:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Material number:	79718845
Sample description:	TOX08907-00
Batch No.:	2010-001067
Nominal content of active substance:	BYI 02960: 200 g/L
Analytical content of active substance:	BYI 02960: 17.0% w/w, 201.0 g/L according to certificate of analysis
Density:	1.175 g/mL at 20°C
Stability of test compound:	Approved until 06/2012 (storage at +2 °C to +30 °C)

2. Test solutions

Test item mixed with:	Quartz sand
Method of preparation:	Sieved soil (2 mm) was treated with either 10 g ground quartz sand/kg dry weight soil (control) or a mixture of quartz sand and BYI 02960 SL 200 G (1.66 µL or 16.59 µL/kg dry weight soil)
Controls:	Water control as negative control

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3. Test soil

Soil nomenclature	Loamy sand
Collection depth	0 to 20 cm
Lot Nr.:	
Source:	Germany/[redacted]
Date of collection:	1 July 2010
Storage temperature:	4 ± 2 °C
Particle size distribution (%) according to DIN:	
clay (< 0.002 mm):	10.3
silt (0.002 - 0.063 mm):	28.3
sand (0.063 - 2.0 mm):	61.4
Soil properties:	
biomass (mg microbial C/kg dry soil):	203
% microbial C of organic carbon:	1.39
% nitrogen	0.135
pH (1mol KCl):	6.88
Cation exchange capacity (meq/100 g dry weight soil):	12.9
History of soil:	
Plant protection products not used since:	2006
Fertilisers not used since:	2006
Crops:	uncultivated

B. Study design and methods

1. In life dates September 9 to October 8, 2010

2. Design of biological test

A loamy sand soil was exposed for 28 days to 1.66 µL and 16.59 µL test item/kg dry weight soil (application rates were equivalent to 1.244 L and 12.44 L test item/ha). 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 bottle (brown glass bottles, 500 mL) filled with 350 g dry weight test soil. The test was conducted with 3 replicates per treatment level. The test was conducted at 20 ± 2 °C.

3. Observation and measurements

At day 0, and after 7, 14 and 28 days of incubation subsamples (moist samples; equivalent to 25 g dry weight) were amended with 2000 mg glucose/kg dry weight. The carbon-dioxide production was measured with a gas analyzer (Wösthoff Co., Bochum, Germany) and the quantities of carbon dioxide released per hour per kg dry weight soil were measured for at least 12 hours.

4. Statistical analysis

All calculations were performed using Microsoft Excel 2003.

The percentage differences in the quantities of CO_2/h/kg dry weight soil formed between control soils and treated soils were expressed as absolute values and determined as follows:

((sum of treatment – sum of control)/sum of control) x 100 % = % difference.

Homogeneity of variances was determined by Cochran's Test, α = 0.05.

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Depending on the results the appropriate T-tests were performed. In the T-test the sum of 12 hours of the values of CO2/h/kg dry weight from control soils and treated soils were compared. The statistical calculations were carried out using ToxRatPro 2.09 (Ratte 2006).

RESULTS AND DISCUSSION

A. Physical and Chemical Parameters

The soil-pH was 7.01 to 7.02. The water content was 42 to 49% of the maximum water holding capacity.

B. Biological Findings

The deviation from the control did not exceed 25% after 28 days.

Table 10.7.1- 1: Effects of BYI 02960 SL 200 G on carbon turnover of the soil microflora in sandy loam given as deviation from the control

Days after treatment	Application rates µL BYI 02960 SL 200 G/kg dws				
	control	1.66		16.59	
	mg CO_2 /h/kg dws	mg CO_2 /h/kg dws	% of control	mg CO_2 /h/kg dws	% of control
0	147.2±4.0	136.8±0.8	93 *w	140.0±0.9	95 *w
7	197.3±8.5	162.7±1.7	82 *)	159.6±4.5	81 *)
14	163.8±6.3	154.8±3.3	94 n.s.	161.1±4.9	98 n.s.
28	142.5±2.7	133.9±0.5	94 *)	135.4±2.7	95 *)

*) Statistically significant difference to the control (Student t-Test, α= 0.05, two-sided)
n.s. No statistically significant difference to the control (t-test, α= 0.05, two-sided)
*w = Statistically significant difference to the control (Welch-t Test for non-homogeneous variances, two-sided, α = 0.05).

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 sodium chloride conducted 2010 demonstrated that 16 g NaCl/kg dry weight soil had distinct and long-term (> 28 days) influence on microbial mineralization of nitrogen.

E. Biological Endpoints Derived

At the end of the experiment differences in the Carbon Dioxide rates between control soil samples and treated soil samples are ≤25 % and meet the trigger values of the above mentioned guideline for a termination of the study.

CONCLUSION

Even though the 10-fold dose revealed a statistically significant difference to the control at the end of the test the deviation from the control was still below the threshold value recommended by the guideline. It therefore can be concluded that BYI 02960 SL G, should not have an impact on carbon transformation in soils when used at rates up to 12.44 L test item/ha.

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Report:	**KIIIA1 10.7.1/02; [redacted] (2010)**
Title:	BYI 02960 SL 200 G: Determination of effects on nitrogen transformation in soil
Report No:	EBRVP083
Document No:	M-396112-01-2
Guidelines:	**OECD guideline 216, 2000**
Deviations:	**None**
GLP:	**Yes (certified laboratory)**

Executive Summary

BYI 02960 SL 200 G (analysed content of a.i.: 201.0 g/L, 17.1% w/w; Specification No.: 102000021884-01; Batch No.: 2010-001067, master recipe ID: 0102142-001, sample description: TOX 08907-00, density: 1.175 g/mL) was used in the test. The objective of the test was to determine the influence of 1.66 µL and 16.59 µL of the test item/kg dry weight soil, respectively on nitrogen-transformation in an agricultural soil.

A loamy sand soil was exposed for 28 d to 1.66 µL and 16.59 µL test item/kg dry weight soil, respectively (application rates were equivalent to 1.244 L and 12.44 L test item/ha, respectively).

Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.

The deviation from the control did not exceed 25% after 28 days. BYI 02960 SL 200 G has negligible effects on nitrogen turnover of soil microflora when used at rates up to 12.449 L test item/ha.

MATERIAL AND METHODS

A. Materials

1. Test material

Test item:	BYI 02960 SL 200
Specification No.:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Material number:	79718845
Sample description:	TOX 08907-00
Batch No.:	2010-001067
Nominal content of active substance:	BYI 02960: 200 g/L
Analytical content of active substance:	BYI 02960: 17.0% w/w, 201.0 g/L according to certificate of analysis
Density:	1.175 g/mL at 20°C
Stability of test compound:	Approved until 14.06.2012 (storage at +2 °C to +30 °C)

2. Vehicle and/or positive control

Test item mixed with:	Quartz sand
Method of preparation:	Sieved soil (2 mm) was treated with either 10 g ground quartz sand/kg dry weight soil
Controls:	Water control as negative control

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3. Test soil

Soil nomenclature:	Loamy sand
Collection depth:	0 to 20 cm
Lot Nr.:	
Source:	Germany/[redacted]
Date of collection:	1 July 2010
Storage temperature:	4 ± 2 °C
Particle size distribution (%) according to DIN:	
clay (< 0.002 mm):	10.3
silt (0.002 - 0.063 mm):	28.3
sand (0.063 - 2.0 mm):	61.4
Soil properties:	
biomass (mg microbial C/kg dry soil):	203
% microbial C of organic carbon:	1.39
% nitrogen	0.135
pH (1mol KCl):	6.88
Cation exchange capacity (meq/100 g dry weight soil):	12.9
History of soil:	
Plant protection products not used since:	2006
Fertilisers not used since:	2006
Crops:	uncultivated

B. Study design and methods

1. In life dates September 9 to October 28, 2010

2. Design of biological test

A loamy sand soil was exposed for 28 d to 1.66 µL and 16.59 µL test item/kg dry weight soil. Application rates were equivalent to 1.244 L and 12.44 L test item/ha. Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation. The nitrogen turnover 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 dry weight test soil. The test was conducted with 3 replicates per treatment level. The test was conducted at 20 ± 2°C.

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 + Lubbe Autoanalyzer 3.

4. Statistical analysis

All calculations were performed using Microsoft 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, α = 0.05.

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Depending on the results the appropriate T-tests were performed. In the T-test, the values of nitrate-N/kg dry weight soil/day from control soils and treated soils were compared. The statistical calculations were carried out using ToxRatPro 2.09 (Ratte 2002).

RESULTS AND DISCUSSION

A. Physical and Chemical Parameters

The soil-pH was 7.24 – 7.27. The water content was 40 to 50% of the maximum water holding capacity.

B. Biological Findings

The deviation from the control did not exceed 25% after 28 days.

Table 10.7.1- 2: Effects of BYI 02960 SL 200 G on nitrogen turnover of the soil microflora in loamy sand given as deviation from the control

Time Interval (days)	Application rates									
	BYI 02960 SL 200 G									
	control			1.66 µL/kg dws				16.59 µL/kg dws		
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	-1.13	±	0.04	-1.25	±	0.03	**11** n.s.	-1.01	±	0.09	**10** n.s.
7-14	1.35	±	0.05	1.28	±	0.26	**5** n.s.w	1.50	±	0.08	**11** n.s.w
14-28	0.99	±	0.09	1.04	±	0.10	**5** n.s.	1.09	±	0.04	**11** n.s.

1) Rate: Nitrate-N in mg/kg dry weight soil/time interval/day, mean of 3 replicates and standard deviation

n.s. = No statistically significant difference to the control (Student-t Test, two-sided, α = 0.05).

n.s.w = No statistically significant difference to the control (Welch-t Test for non-homogeneous variances, two-sided, α = 0.05).

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 sodium chloride conducted 2010 demonstrated that 16 g NaCl/kg dry weight soil had distinct and long-term (> 28 days) influence on microbial mineralization of nitrogen.

E. Biological Endpoints Derived

At the end of the experiment differences in the nitrogen rates between control soil samples and treated soil samples are <25 % and meet the trigger values of the above mentioned guideline for a termination of the study.

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CONCLUSION

During the 28-day test, in a soil supplemented with lucerne-grass-green-meal (5 g/kg), it was found that 1.66 µL test item/kg dry weight soil (equivalent to 1.244 L test item/ha) and the 10-fold dose of the test item had no relevant influence on nitrogen transformation in a loamy sand. It can therefore be concluded that BYI 02960 SL 200 G should not have an impact on nitrogen transformation in soils if used at rates up to 12.449 L test item/ha.

IIIA1 10.7.2 Further testing to investigate impact on soil microbial activity

According to the previous results (see Point 10.7.), no further laboratory testing on soil non-target micro-organisms was considered necessary.

IIIA1 10.8 Effects on non-target plants

IIIA1 10.8.1 Effects on non-target terrestrial plants

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area. Spray drift from the treated areas may lead to residues of a product in off-crop areas.

In the case of a non-herbicide, screening results and/or Tier 1 studies give first information about the likelihood for terrestrial plant effects. The risk can be considered acceptable if there are no data indicating more than 50% phytotoxic effect at the maximum application rate.

Seedling emergence ([redacted] (2010), M-397727-01-2, KIIIA1 10.8.1.3/01) and vegetative vigour studies [redacted] (2010), M-397734-01-2, KIIIA1 10.8.1.2/01) have been conducted with BYI 02960 SL 200 G following OECD testing guidelines 208 and 227, respectively (see Annex Points IIIA1 10.8.1.3 and 10.8.1.2). Each study included 11 species which were tested at the maximum application rate of 410 g a.i./ha.

In the case of BYI 02960 SL 200 G neither the tier 1 seedling emergence nor the vegetative vigour studies showed phytotoxic effects >50% at the maximum application rate of 410 g a.i./ha.

Therefore, it can be concluded that effects of the product on non-target terrestrial plants in off-crop areas are unlikely and no further risk assessment is necessary.

IIIA1 10.8.1.1 Seed germination

Please refer to Point IIIA 10.8.1.3.

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IIIA1 10.8.1.2 Vegetative vigour

Report:	**KIIIA1 10.8.1.2/01; [redacted] H., 2010**
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)**
Deviations:	**None**
GLP:	**Yes (certified laboratory)**

The full summary of this study is filed in the Annex II document, as it is a core requirement (see KIIA 8.12/01). However, a short overview is presented below.

Executive summary

The purpose of this specific study is to evaluate the potential side effects of BYI 02960 SL 200 g/L (Sample description: TOX08854-00; Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 102000021884-01) on the vegetative vigour of eleven non-target terrestrial plant species following a post-emergence 410 g a.i./ha application of the product onto the foliage of plants.

A total of eleven species were tested in this vegetative vigour test including seven dicotyledonous and four monocotyledonous species representing nine plant families.

At the 2-4 leaf stage, plants (except *Allium cepa*, which was treated at the 1-2 leaf stage) were sprayed once with BYI 02960 SL 200 g/L at an application rate of 410 g a.i./ha and a volume rate of 200 L/ha.

Each pot (replicate) contained 4 plants and there were 32 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, 14 and 21 days after application and assessments were made against the water treated controls. The study was terminated 21 days after application.

Following a foliar application of BYI 02960 SL 200 g/L applied at 410 g a.i./ha (corresponding to 2.4 kg product/ha) to eleven terrestrial non-target 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.

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IIIA1 10.8.1.3 Seedling emergence

Report:	**KIIIA1 10.8.1.3/01; [redacted] H., 2010**
Title:	BYI 02960 SL 200 g/L – Effects on the seedling emergence and growth of eleven species of non-target terrestrial plants (Tier 1)
Report No:	SE10/001
Document No:	M-397727-01-2
Guidelines:	**OPPTS 850.4100 (1996);** **OECD Guideline 208 (2006)**
Deviations:	**None**
GLP:	**Yes (certified laboratory)**

The full summary of this study is filed in the Annex II document, as it is a core requirement (see KIIA 8.12/02). However, a short overview is presented below.

Executive summary

The purpose of this specific study is to evaluate the potential phytotoxic effects of BYI 02960 SL 200 g/L (Sample description: TOX 08854-00; Batch ID: 2009-001253; Material No.: 79718845; Specification No.: 102000021884-01) 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 410 g a.i./ha.

A total of eleven species were tested in this seedling emergence and growth test including seven dicotyledonous 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 de-ionized 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 eleven terrestrial non-target plant species, no adverse effects on emergence, seedling survival, 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.

IIIA1 10.8.1.4 Terrestrial field testing

Further studies were not considered necessary.

IIIA1 10.8.2 Effects on non-target aquatic plants

The toxicological spectrum of the product as well as the single active substances towards aquatic plants is presented under the Point 10.2. The risk assessment for *Lemna* is presented under point 10.2.1.11.

IIIA1 10.8.2.1 Aquatic plant growth – Lemna

Tests 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 under Regulation (EC) 1107/2009; however for registrations in other regions (US and Canada) data must be provided for the macrophyte *Lemna*, therefore a 7 day growth inhibition test is available which is presented in the Annex II document (see KIIA 8.6/01).

IIIA1 10.8.2.2 Aquatic field testing

The spectrum of the biological activity of the product is well represented by the results and the risk assessments in Point 10.2. Therefore, further studies are not considered necessary.

IIIA1 10.9 Effects on other non-target organisms believed to be at risk

The spectrum of the biological activity of the product is well represented by the results and the risk assessments in Point 10.2 to 10.8 of this dossier. Therefore, further data from biological primary screening or other preliminary tests are not considered relevant for the risk assessment.

IIIA1 10.9.1 Summary of preliminary data: biological activity & dose range finding

Not relevant. See statement provided under Point 10.9.

IIIA1 10.9.2 Assessment of relevance to potential impact on non-target species

Not relevant. See statement provided under Point 10.9.

IIIA1 10.10 Other/special studies

The spectrum of the biological activity of the product is well represented by the results and the risk assessments in Point 10.2 to 10.8 of this dossier. Therefore, further data from biological primary screening or other preliminary tests are not considered relevant for the risk assessment.

IIIA1 10.10.1 Other/special studies - laboratory studies

Not relevant. See statement provided under Point 10.10.

IIIA1 10.10.2 Other/special studies - field studies

Not relevant. See statement provided under Point 10.10.

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IIIA1 10.11 Summary and evaluation of points IIIA1 9 and IIIA1 10.1 to 10.10

IIIA1 10.11.1 Predicted distribution and fate in the environment and time courses

The distribution and fate of the active substances was assessed in laboratory studies, field studies and predictive modelling in the soil, aquatic and air, are summarised in Section 5 Point 9.

IIIA1 10.11.2 Non-target species at risk and extent of potential exposure

A summary of the respective document chapters, conclusions and potential risk mitigation measures is given in the following text:

Terrestrial vertebrates

The Tier 1 risk assessment for birds showed that all toxicity-to-exposure-ratios (TER) exceed the *a-priori* acceptability criteria of the EU Regulation 1107/2009. Thus, the risk for effects on birds from exposure to BYI 02960 after use of the product as described in this dossier can be considered as low and acceptable.

The Tier 1 risk assessment for mammals also resulted in TER values in excess of the *a-priori* acceptability criteria for all acute or reproductive scenarios, except for small herbivorous mammals where a refined reproductive risk assessment (Tier 2) was conducted. Elements of this refined risk assessment included measured residue decline data, a review of the toxicological profile with regard to ecological relevance for wild mammal populations, the evaluation of literature and field study information on the relevance of hop yards and lettuce fields as habitat for vole populations, and the known resilience of the species. Both the qualitative considerations and the quantitative assessment (Tier 2 TER_{LT} values > 5) allow the conclusion that the risk for wild mammals including populations of small herbivorous mammals can be considered as low and acceptable. Risk to birds and mammals from exposure via drinking water was considered to be low. The risk of adverse effects on vertebrates from exposure to metabolites is considered to be low and covered by the risk assessment for the parent compound.

No risk via secondary poisoning is to be expected from the use of the product according to the intended use pattern.

Aquatic organisms

The TER values for fish, aquatic invertebrates, algae and *Lemna* based on FOCUS Step 2 PEC_{SW} values are in correspondence with the trigger values indicating that the use of the product does not raise any direct concern when applied at the recommended rate. All Tier 1 TER values for the metabolites meet the a-priori acceptability criteria. Thus, no unacceptable adverse effects on aquatic organisms are to be expected from the exposure to these metabolites.

Aquatic insects are the most sensitive taxonomic group as indicated by the effects observed with *Chironomus riparius*, safe use for aquatic insects can be demonstrated at FOCUS Step 3 in one scenario and at FOCUS Step 4 considering potential mitigation measures for further scenarios.

Honey bees

Overall, the laboratory database shows that BYI 02960 does not exhibit delayed or chronic effects, either in adult bees or in honey bee larvae. BYI 02960 metabolites are virtually non-toxic to honey

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bees and there is no indication that BYI 02960 metabolites are of any higher toxicity regarding potentially delayed or chronic effects than the parent compound. These laboratory findings have been consistently confirmed by in total six independent semi-field tunnel studies in the highly bee attractive surrogate crop *Phacelia tanacetifolia*. As such, it can be concluded that BYI 02960 can be applied at foliar application rates of up to and including 200 g a.i./ha, even to bee-attractive, full-flowering crops during honey bees actively foraging, without adverse effects on honey bees, honey bee brood and honey bee colonies.

Terrestrial non-target arthropods

The refined NTA risk assessment indicated based on the results of the aged residue studies and the results of the NTA full fauna off-field studies that no unacceptable adverse effects on non-target arthropods are to be expected for the in- or off-field habitats following the use of the product according to the proposed use pattern.

Earthworms and other soil non-target macro-organisms

As has been demonstrated by laboratory acute and chronic studies and an earthworm field study, no unacceptable effects on earthworms are to be expected from the application of the product according to the proposed use pattern.

Chronic laboratory tests with *Folsomia candida* and *Hypoaspis aculeifer* also indicate that no adverse effects on other soil non-target macro-organisms are to be expected from the use of the product.

Non-target soil micro-organisms

The risk assessment indicates that no adverse effects on soil micro-organisms are to be expected when the product is applied according to the proposed use pattern.

Terrestrial non-target plants

The effect of BYI 02960 SL200 on seedling emergence and vegetative vigour of terrestrial non-target plants has been tested in course of two Tier-I limit tests with a single rate of 410 g a.i./ha. This rate covers multiple applications of lower rates multiplied with a MAF (multiple application factor). At this rate no inhibitory effect above 20% was observed in any of the ten species tested. In the off-crop area non-target plants are exposed to spray-drift only. It can be concluded that terrestrial non-target plants are not at risk when BYI 02960 SL200 is applied at rates recommended according to good agricultural practice.

IIIA1 10.11.3 Short and long term risks for non-target organisms

Please refer to Point IIIA 10.11.2.

IIIA1 10.11.4 Risk of fish kills and fatalities in large vertebrates

According to the aquatic risk assessment provided under Point 10.2, application of the product according to the proposed use will not result in unacceptable adverse effects for fish.

Based on the information presented under Points 10.1 and 10.3, it is most unlikely that unacceptable risks will occur in large vertebrates and terrestrial predators when the product is used in accordance with the label recommendations.

IIIA1 10.11.5 Precautions necessary to avoid or minimize contamination

No unacceptable risk to non-target organisms is to be expected from the application of the product when following the proposed risk mitigation for aquatic organisms.

Further information is given under Point 10.11.2 of this document.

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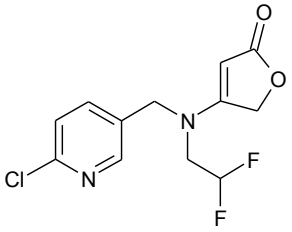
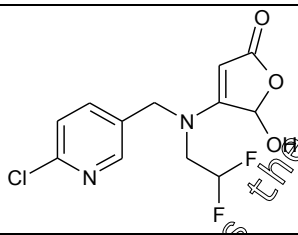
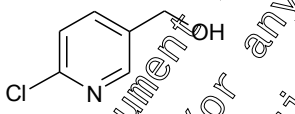
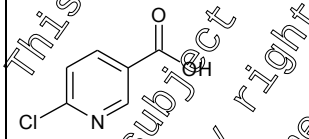
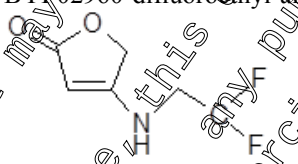
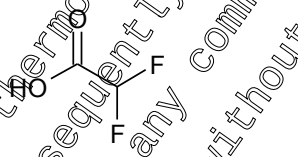
List of BYI 02960 metabolites included in this section

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.

	Name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
a.s.	**BYF 02960 (parent compound)** 	C_{12} H_{11} Cl F_2 N_2 O_2 288.68 g/mol Flupyradifurone	all matrices
M03	BYI 02960-OH		
		C12 H11 Cl F2 N2 O3 304.68 g/mol BYI 02960-hydroxy BCS-CQ74364	Animal, Plant:
M21	BYI 02960-CHMP		
		C6 H6 Cl N O 143.57 g/mol 6-CPA (6-chloro-picrylalcohol) BCS-AA52175	Plant:
M27	6-CNA 	C_6 H_4 Cl N O_2 157.56 g/mol 6-chloronicotinic acid IC-0 (in reports from Nippon Soda Co. Ltd.) BYI 02960-6-CNA BCS-AA35572	Animal, Plant: Environment Aerobic soil (major)
M34	BYI 02960-difluoroethyl-amino-furanone 	C_6 H_7 F_2 N O_2 163.12 g/mol DFEAF	Animal, Plant
M44	DFA 	C_2 H_2 F_2 O_2 96.03 g/mol difluoroacetic acid BYI 02960-DFA BCS-AA56716 (In aquatic studies, tested as sodium difluoroacetate (Na-salt of difluoroacetic acid) (code: BCS-AB60481)	Animal, Plant: Environment Aerobic Soil (major) Aerobic water/Sediment (major)

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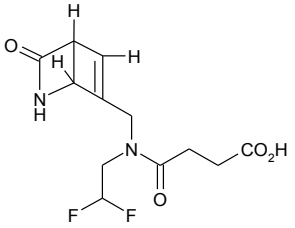
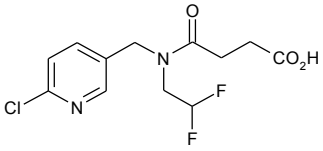
	Name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M47	**BYI 02960-azabicyclosuccinamide**		
		$C_{12}H_{14}F_2N_2O_4$ 288.25 g/mol BCS-CS64875 (Tested as BYI 02960-azabicyclosuccinamide Na-Salt, BCS-CU93236)	Environment Water – aquatic photolysis (major)
M48	**BYI 02960-succinamide**		
		$C_{12}H_{13}ClF_2N_2O_3$ 306.69 g/mol BCS-CR74729	Environment Water – Aquatic photolysis (major)