whistling

Document Title

Tier 2 Summary of the Ecotoxicological Studies on the Active Substance for

Spirotetramat (BYI 08330)

Data Requirements

Directive 91/414/EEC
Regulatory Directive 98-04/Canada/PMRA
OPPTS guidelines/US/EPA

Annex IIA
Section 6, Point 8
Document M

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

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Author(s)

[redacted]

Bayer CropScience

[redacted]

OWNERSHIP STATEMENT

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IIA 8 Ecotoxicological Studies on the Active Substance

In the following, summaries of the ecotoxicological studies are presented that have been conducted to characterise the active substance spirotetramat. An assessment of ecotoxicological data is only possible in connection with the label recommendations and the environmental exposure resulting from the use according to good agricultural practice. Therefore the assessment is made in the Annex III dossier of the formulation.

Compound synonyms:

The chemical code for spirotetramat is BYI 08330, which is used in most studies of this dossier.

IIA 8.1 Avian toxicity

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

Report:	**KIIA 8.1.1/01, [redacted]; 2003**
Title:	Acute Oral Toxicity for Bobwhite Quail (*Colinus virginianus*) for the Test Item BYI 08330 (tech.). Date: 2003-10-29, amended 2003-11-10
Organisation:	[redacted], Germany
Report No.:	BAR/LD 047; M-115911-02-2
Publication:	unpublished
Dates of experimental work:	August 19, 2003 - September 03, 2003
Guidelines:	US-EPA Pesticide Assessment Guidelines-Subdivision E, § 71-1 "Avian single-dose oral LD_{50} test" (October 1982), in consideration of US-EPA Ecological Effects Test Guideline "OPPTS 850.2100 Avian Acute Oral Toxicity Test" (April 1996). MAFF (UK)-Working Document No. 7/5: "Evaluating the acute oral and short-term cumulative oral toxicity of pesticides to birds."
Deviations:	No deviations from EPA § 71-1.
GLP:	yes (certified laboratory)

Executive summary

The test item was administered as a single oral administration in gelatine capsules to singly housed adult Bobwhite quails. 10 birds per treatment level (5 males + 5 females) were exposed to 0 mg a.s./kg bw (control), 500 mg a.s./kg bw, 1000 mg a.s./kg bw and 2000 mg a.s./kg bw. The dosing was followed by a subsequent observation period of 14 days. The birds were observed on mortality, behavioural impacts and effects on food consumption and body weights. Additionally, gross pathological changes were determined by necropsies. Body weight was measured on day -1, day +7 and on day 14 (test termination).
The LD_{50} for adult Bobwhite quails was higher than 2000 mg a.s./kg bw.

MATERIAL AND METHODS

A Materials

1. Test material **Spirotetramat (BYI 08330), tech.**

Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014 (mixed batch)** **Tox no.: TOX06344-00**
Purity	**97.5%**
Stability of test compound	**Approved until 2003-12-23 when stored at room conditions**
2. Vehicle and/or positive control	**Gelatine capsule, Size No.00 (one per bird) disintegration time:5 Minutes (US-federal standard No.285A-0,5% HCL)**
3. Test animals	
Species	**Bobwhite quail (*Colinus virginianus*)**
Age	**Sexually mature**
Source	**[redacted], Germany**
Acclimation period	**15 hours starvation prior to oral administration**
Environmental conditions	
Temperature	**18.6 - 21.1°C**
Photoperiod	**10 h light (daylight visual spectrum) / 14 h dark**

B Study design and methods

1. In life dates **August 19, 2003 - September 03, 2003**

2. Experimental treatments

Pen reared, sexually mature Bobwhite quail (*Colinus virginianus*) were purchased from a commercial breeder. All birds appeared phenotypically similar to birds from wild populations. They were approaching their first breeding season and had not been used in previous testing. After arrival, all birds were moved to their test facilities. Birds which were injured or did not appear healthy were excluded.

A standard diet for quails (type: Alleinfutter fur Wachteln und Enten G4, Batch No.: 32/03, Art. No. 3958.9.25, of the company "[redacted]", CH-[redacted], Switzerland) and drinking water (Monheim City municipal water) was supplied *ad libitum* prior and throughout the study, except a 15 hours starvation period immediately before application. The food was analysed for contaminants.

The birds were singly housed in stainless steel wire cages (38 x 25 cm x 23 cm) which were placed indoors in four superposed rows. The front of each cage was equipped with a polyethylene-feeder.

Five male and five female birds were allocated randomly to each of the three treatment groups (500, 1000 and 2000 mg a.s./kg bw) and one control group. In the same way, the cage-blocks of each testing group were randomly assigned to the testing-units in a randomized block design. All birds were identified by numbered and coloured leg bands. One day prior to dosing, the body weights of the birds were

determined for the calculation of the individual test substance amounts. At the end of the study all surviving birds were sacrificed by CO_2 asphyxiation.

3. Observations

Observations on mortality and signs of intoxication (e.g. type, severity and duration of changes in behaviour) were made hourly on the day of dosing and at least once on weekdays throughout the 14 days observation period.

The individual body weights of the animals for each dose and control group were recorded prior to test initiation (day -1), on study day 7 and on day 14 (test termination).

Average food consumption was determined of each animal per day during the time periods from days 0 to 3, days 3 to 7 and from day 7 to 14. Therefore, on study days 3 and 7 all remaining food was replaced by fresh food. The removed food was weighed for determination of the food consumption.

Gross necropsies were carried out on all premature deaths and on all survivors at the end of the study.

Statistical evaluation on body weights, food consumption and LD_{50} calculation was considered not necessary due to the clear results of the study.

RESULTS AND DISCUSSION

A. Findings

Acute oral toxicity to birds

Test substance	Spirotetramat tech.
Test object	Bobwhite quail f/m
LD_{50} [mg a.s./kg bw]	> 2000
Lowest Lethal Dose (LDD) [mg a.s./kg bw]	2000

B. Observations

Prematurely dead:

At the highest test dose 2000 mg a.s./kg body weight, 2 of 10 birds died prematurely (1 male on day +10, 1female at day +6). The birds suffered on diarrhoea from day 1 on until their death, combined with other signs of impairment (fluffed feathers, ptosis, apathy, discoordinated movements). At gross necropsy the male showed no specific symptoms while the female showed different severe impacts on the digestive tract, including purulence.

Survivors:

The surviving birds at 2000 mg a.s./kg bw showed soft excrements and diarrhoea after the application, but were completely recovered on day 7. Reduced food consumption was observed until day +7.

Diarrhoea and soft excrements were also observed at 500 and 1000 mg a.s./kg bw. These symptoms ceased on day 3 on the 500 mg a.s./kg bw group and on day 10 in the 1000 mg test group. Observed symptoms of one male in the 1000 mg a.s./kg bw test group (fluffed

feathers, apathy, injured at one foot) were not considered to be treatment related. Reduced food consumption was observed in the 500 and 1000 mg a.s./kg bw group until day +3.
All survivors were free of symptoms at test termination.
Gross necropsy did not reveal specific symptoms, but some individual changes, which are considered to be within natural variation.

CONCLUSION

The LD_{50} for adult Bobwhite quails was higher than 2000 mg a.s./kg bw.

Report:	**KIIA 8.1.1/02, [redacted]; 2008a**
Title:	Acute oral toxicity test (LD_{50}) with the test substance BYI 08330 (tech.) for chicken (*Gallus gallus domesticus*) Date: 2008-06-25
Organisation:	[redacted], Germany
Report No.:	BAR/LD 088; M-303556-01-1
Publication:	unpublished
Dates of experimental work:	March 3, 2008 – April 8, 2008
Guidelines:	US-EPA Pesticide Assessment Guidelines-Subdivision E, § 71-1 "Avian single-dose oral LD_{50} test" (October 1982), in consideration of US-EPA Ecological Effects Test Guideline "OPPTS 850.2100 Avian Acute Oral Toxicity Test" (April 1996). MAFF (UK)-Working Document No. 7/5: "Evaluating the acute oral and short-term cumulative oral toxicity of pesticides to birds."
Deviations:	No major deviations
GLP:	yes

Executive summary

The test item was administered as a single oral administration in gelatine capsules to individually housed subadult female chicken. 10 birds per treatment level were exposed to 0 mg a.s./kg bw (control), 1000 mg a.s./kg bw and 2000 mg a.s./kg bw. The dosing was followed by a subsequent observation period of 21 days. The birds were observed on mortality, behavioural impacts and effects on food consumption and body weights. Additionally, gross pathological changes were determined by necropsies.

Body weight was measured on day -1, day +7, day 14 and on day +21 (test termination).

The LD_{50} for subadult chicken was higher than 2000 mg a.s./kg bw. The lowest lethal dose (LLD) was > 2000 mg a.s./kg bw.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330), tech.**
Description	**Light beige powder**
Lot/batch No.	**Batch no.: 08045/0014 (mixed batch)** **Tox no.: TOX 07425-02**
Purity	**98.1%**
Stability of test compound	**Approved until 2008-05-03 when stored at room conditions**
2. Vehicle and/or positive control	**Gelatine capsule, Size No.00 (up to four per bird)** **disintegration time:5 Minutes (US-federal standard No.285A-0,5% HCL)**
3. Test animals	
Species	**Chicken (*Gallus gallus domesticus*)**
Age	**14 weeks old**

Source	[redacted]
Acclimation period	**14 days under test conditions in the test facility**
Environmental conditions	
Temperature	**16.8 - 26.5°C**
Photoperiod	**10 h light (daylight visual spectrum) / 14 h dark**

B Study design and methods

1. In life dates **March 03, 2008 – April 08, 2008**

2. Experimental treatments

Subadult female chicken (*Gallus gallus domesticus*) were purchased from a commercial breeder. The birds were 14 weeks old. 10 females per group were allocated to each of the 2 treatment groups (1000 and 2000 mg a.s./kg bw) and one control group. All birds were identified by numbered and coloured leg bands. Acclimation time lasted 14 days. One day prior to dosing, the body weights of the birds were determined for the calculation of the individual test-substance amounts. The birds were starved for 16 hours prior to oral administration of the test item. At all other times (acclimation time and post exposure period) the birds had free access to food.

The birds were fed with a standard rearing diet for chicken (type: [redacted], [redacted] e.v., Batch No. 3168309, of the company [redacted], D-[redacted]). Drinking water (Monheim City municipal water) was supplied *ad libitum* prior and throughout the study.

The birds were housed in stainless steel wire cages (44 x 33 cm x 43 cm) which were placed indoors in three superposed rows. The front of each cage was equipped with a steel-feeder.

3. Observations

Observations of mortality and signs of intoxication were made continuously during the first hour and approximately hourly on the day of dosing and at least once work-daily throughout the post exposure observation period (body weights were recorded prior to the test initiation (day -1), then weekly (day +7, day +14) and on test termination (day +21).

On study days 3, 7, 14 (except day 21, only reweigh) all remaining food (per hen) was replaced by fresh food after cleaning. The removed food was reweighed for determination of the food consumption. At the end of the study all surviving birds were sacrificed by CO_2 asphyxiation.

If there had been toxic symptoms at the end of the 21 day post-exposure period, the study would have been prolonged until no symptoms were visible.

Because no case of mortality occurred slope and confidence interval could not be calculated using the Probit-Test (Toxcalc v 1.0). For statistical evaluation of possible treatment related effects on body weights, the data was processed as unpaired comparisons of each treatment level with untreated control.

RESULTS AND DISCUSSION

A. Findings

Acute oral toxicity to birds

Test substance	Spirotetramat (BYI 08330)
Test object	Chicken f
LD_{50} [mg a.s./kg bw]	> 2000
Lowest Lethal Dose (LDD) [mg a.s./kg bw]	> 2000

B. Observations

Mortality and behaviour

No mortality occurred. In both test concentration and in the control all birds showed diarrhoea, excretion of uric acid and soft excrement. At 1000 mg a.s./kg bw additionally one bird showed reduced vigilance on day 7 and in the 2000 mg a.s./kg bw group some birds also showed uncoordinated movements, reduced vigilance and fluffed feathers in the first week after dosing.

Body weight development

The mean body weight of the control birds increased continuously up to the end of the study. At 1000 mg a.s./kg b.w., the body weight was transiently slightly reduced after dosing, but after 2 weeks the body weight in this group was again similar to the control. At 2000 mg a.s. /kg b.w. the body weight loss was more pronounced, but the body weight of the birds increased from day 7 on. At the end of the study, it was in the range of the control.

Food consumption

During the first week after dosing, the food consumption of the treated groups was apparently reduced if compared with the control. This effect was more obvious at 2000 mg a.s./kg bw than at 1000 mg a.s./kg bw and can explain the body weight loss as described above. From day +7 on, both treated groups consumed more food than the control.

Gross pathology

Two birds of the 2000 mg a.s. group showed an enlarged gall bladder. These findings were considered to be within natural variation.

CONCLUSION

The LD_{50} for subadult chicken was higher than 2000 mg a.s./kg bw. The lowest lethal dose (LLD) was > 2000 mg a.s./kg bw.

Report:	**KIIA 8.1.1/03, [redacted].; 2009**
Title:	BYI08330: An acute oral toxicity study with the canary (*Serinus canaria*)
	Date: 2009-05-19
Organisation:	[redacted], USA
Report No.:	EBFNP146; M-348059-01-1
Publication:	unpublished
Dates of experimental work:	March 20, 2009 to April 03, 2009
Guidelines:	US-EPA Pesticide Assessment Guidelines-Subdivision E, § 71-1 "Avian single-dose oral LD_{50} test" (October 1982), in consideration of US-EPA Ecological Effects Test Guideline "OPPTS 850.2100 Avian Acute Oral Toxicity Test" (April 1996)
Deviations:	No major deviations
GLP:	yes

Executive summary

The test item was administered as a single oral administration in gelatine capsules to adult canaries housed by pair. 10 birds per treatment level (five males, five females) were exposed to 0 (control), 125, 250, 500, 1000 and 2000 mg a.s./kg bw (geometric series of dosage groups). The dosing was followed by a subsequent observation period of 14 days. The birds were observed on mortality, behavioural impacts and effects on food consumption and body weights. Additionally, gross pathological changes were determined by necropsies.

Body weight was measured at the initiation of the test and on day +3, day +7 and day 14 (test termination).

The LD_{50} for adult canaries was higher than 2000 mg a.s./kg bw. The no-observed-effect-level (NOEL) was 500 mg a.s./kg bw.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330), tech.**
Description	**Light beige powder**
Lot/batch No.	**Batch no.: 08045/0014 (mix-batch)**
	Tox no: 07425-03
Purity	**98.1%**
Stability of test compound	**Approved until 2010-04-24 (storage at +25 ± 5°C)**
2. Vehicle and/or positive control	**Gelatine capsule, Size No.4 (one per bird)**
	Control birds received an empty capsule
3. Test animals	
Species	**Canary (*Serinus canaria*)**
Age	**adult**
Source	**[redacted] MD**
Acclimation period	**16 days - fasting: 2 hours prior to start of test**
Environmental conditions	
Temperature	**22.4 ± 1.3°C**

Photoperiod	8 h light / 16 h dark

B Study design and methods

1. In life dates March 20, 2009 to April 03, 2009

2. Experimental treatments

Adult canaries (*Serinus canaria*) were purchased from a commercial breeder. Five pens (each with 1 male and 1 female) per group were allocated to each of the 5 treatment groups (125, 250, 500, 1000 and 2000 mg a.s./kg bw) and one control group. Individual birds were identified by numbered and coloured leg bands. Acclimation time lasted 16 days. One day prior to dosing, the body weights of the birds were determined for the calculation of the individual test-substance amounts. The birds were starved for at least 2 hours prior to oral administration of the test item. At all other times (acclimation time and post exposure period) the birds had free access to food.

The birds were fed with commercially available canary food, millet and/or a ration consisting of small-grained seeds. Drinking water (Easton public water supply) was supplied *ad libitum* prior and throughout the study.

The birds were housed in coated wire cages (29 cm x 26 cm x 31 cm) which were placed indoors in batteries; sidewalls consisted of plastic.

3. Observations

During acclimation, all birds were observed daily. Birds exhibiting abnormal behavior or physical injury were not used. Following dosing, multiple observations were performed on Day 0 of the test, with particular attention being paid for signs of regurgitation. From test initiation until termination, all birds were observed at least twice daily. A record was maintained of all mortality, signs of toxicity, and abnormal behavior.

Body weights were measured individually one day prior initiation of the test (to allow time for capsule preparation) and on Days 3, 7 and 14 of the test.

A gross necropsy was performed on the one mortality. The gross necropsy included but was not limited to a general examination of the exterior of the bird and an examination of the thoracic and abdominal cavities, including cardiovascular and respiratory systems, liver, spleen, gastro-intestinal tract and urogenital system.

RESULTS AND DISCUSSION

A. Findings

Acute oral toxicity to birds

Test substance	Spirotetramat (BYI 08330)
Test object	Canary f/m
LD_{50} [mg a.s./kg bw]	> 2000
No-observed-effect- level(NOEL) [mg a.s./kg bw]	500

B. Observations

Mortality and clinical observations

There were no mortalities in the control group and all birds in the control group were normal in appearance and behavior for the duration of the test. In addition, there were no mortalities at the 125, 250, 500 or 2000 mg a.s./kg dosage levels. There was 10% mortality (1 of 10) at the 1000 mg a.s./kg dosage level occurring within the first hour after dosing. No indications of regurgitation were noted in the control group or any of the treatment groups.

In the 125 mg a.s./kg dosage group no signs of toxicity were noted. One bird in the 125 mg a.s./kg dosage group was noted with an irritated left eye on Days 7 to 13 of the test. All other birds in the 125 mg a.s./kg dosage group were normal in appearance and behavior for the duration of the test.

All birds in the 250 and 500 mg a.s./kg dosage groups were normal in appearance and behavior for the duration of the test.

In the 1000 mg a.s./kg dosage group, a total of six birds were noted with signs of toxicity on the day of dosing. Signs of toxicity were first noted twenty-nine minutes after the completion of dosing, when one bird was noted with a ruffled appearance and a second bird was noted with convulsions, prostrate posture and loss of righting reflex, followed by death.
One bird continued to be noted with signs of toxicity (ruffled appearance) on the morning of Day 1 and the morning and afternoon of Day 2. All surviving birds had recovered by the morning of Day 3 and were normal in appearance and behaviour for the remainder of the test.

In the 2000 mg a.s./kg dosage group, a total of six birds were noted with signs of toxicity on the day of dosing. Signs of toxicity were first noted twenty minutes after dosing, when three birds were noted with a ruffled appearance. All birds had recovered by the last observation on the day of dosing. One female was noted with a slightly irritated left eye on Day 13 of the test. On the morning of Day 14 of the test this bird was noted with both eyes irritated, a ruffled appearance and as gaping. These observations were not considered to be treatment related but a result of pen-mate aggression. With the exception of this bird, all birds were normal in appearance and behaviour from Day 1 of the test until test termination.

Body weight development

When compared to the control group, there were no apparent treatment related effects upon body weight at any interval for surviving males or females at the 125, 250, 500, 1000 or 2000 mg a.s./kg dosage levels.

Necropsy

A gross necropsy was performed on the one mortality, a female in the 1000 mg a.s./kg dosage level. The bird was noted with a small area of intracranial bleeding, but was otherwise unremarkable.

CONCLUSION

The acute oral LD_{50} value for canary exposed to BYI 08330 as a single oral dose was determined to be greater than 2000 mg a.s./kg, the highest dosage level. The no-observed-effect level was 500 mg a.s./kg. There was 10% mortality (1 of 10) at the 1000 mg a.s./kg dosage level. There were no mortalities in the control or the 125, 250, 500 and 2000 mg a.s./kg dosage levels.

Report:	**KIIA 8.1.1/04, [redacted]; 2008b**
Title:	Acute oral toxicity test (LD50) with the test substance BYI 08330 OD 150B G for chicken (*Gallus gallus domesticus*) Date: 2008-06-25
Organisation:	[redacted], Germany
Report No.:	BAR/LD 089; M-303555-01-1
Publication:	unpublished
Dates of experimental work:	March 3, 2008 – April 8, 2008
Guidelines:	US-EPA Pesticide Assessment Guidelines-Subdivision E, § 71-1 "Avian single-dose oral LD_{50} test" (October 1982), in consideration of US-EPA Ecological Effects Test Guideline "OPPTS 850.2100 Avian Acute Oral Toxicity Test" (April 1996). MAFF (UK)-Working Document No. 7/5c "Evaluating the acute oral and short-term cumulative oral toxicity of pesticides to birds"
Deviations:	No major deviations
GLP:	yes

Executive summary

The test item was administered as a single oral administration in gelatine capsules to individually housed subadult female chicken. 10 birds per treatment level were exposed to 0 mg form./kg bw (control), 1000 mg form./kg bw and 2000 mg form./kg bw. The dosing was followed by a subsequent observation period of 21 days. The birds were observed on mortality, behavioural impacts and effects on food consumption and body weights. Additionally, gross pathological changes were determined by necropsies.

Body weight was measured on day -1, day +7, day 14 and on day +21 (test termination).

No mortality and no signs of intoxication occurred throughout the duration of the test.

Slight reduction of body weights at the 2000 mg formulation/kg bw treatment group.

During the first three days after the exposure the food consumption in the treated group was lower than in the control. Afterwards all groups behaved similarly.

No substance related pathological findings at necropsy.

The LD_{50} for subadult chicken was higher than 2000 mg formulation/kg bw. The lowest lethal dose (LLD) was > 2000 mg formulation/kg bw.

Material and Methods

A Materials

1. Test material	**BYI 08330 OD 150B G**
Description	**Light brown suspension**
Lot/batch No.	**Batch-ID.: 2007-005113** **Tox No.: 07966-00**
Content of A.S.	**15.6% w/w (154.1 g/L)**
Density	**0.988 g/mL**
Stability of test compound	**Approved until 2008-06-01 when stored at room conditions**

2. Vehicle and/or positive control	Gelatine capsule, Size No.00 (up to four per bird) disintegration time:5 Minutes (US-federal standard No.285A-0,5% HCL)
3. Test animals	
Species	Chicken (*Gallus gallus domesticus*)
Age	14 weeks old
Source	[redacted], Germany (Date of supply :2008-03-03)
Acclimation period	14 days under test conditions in the test facility
Environmental conditions	
Temperature	16.8 - 26.5°C
Photoperiod	8 h light, 16 h dark

B Study design and methods

1. In life dates: March 03, 2008 – April 08, 2008

2. Experimental treatments

Subadult female chicken (*Gallus gallus domesticus*) were purchased from a commercial breeder. The birds were 14 weeks old. 10 females per group were allocated to each of the 2 treatment groups (1000 and 2000 mg a.s./kg bw) and the one control group. All birds were identified by numbered and coloured leg bands. Acclimation time lasted 14 days. One day prior to dosing, the body weights of the birds were determined for the calculation of the individual test-substance amounts. The birds were starved for 16 hours prior to oral administration of the test item. At all other times (acclimation time and post exposure period) the birds had free access to food.

The birds were fed with a standard rearing diet for chicken (type: [redacted], [redacted] e.v., Batch No. 3168309, of the company [redacted], [redacted]). Drinking water (Monheim City municipal water) was supplied *ad libitum* prior and throughout the study.

The birds were housed in stainless steel wire cages (44 x 33 cm x 43 cm) which were placed indoors in three superposed rows. The front of each cage was equipped with a steel-feeder.

3. Observations

Observations of mortality and signs of intoxication were made continuously during the first hour and approximately hourly on the day of dosing and at least once work-daily throughout the post exposure observation period (body weights were

recorded prior to the test initiation (day -1), then weekly (day +7, day +14) and on test termination (day +21).
On study days 3, 7, 14 (except day 21, only reweigh) all remaining food (per hen) was replaced by fresh food after cleaning. The removed food was reweighed for determination of the food consumption. At the end of the study all surviving birds were sacrificed by CO_2 asphyxiation.
If there had been toxic symptoms at the end of the 21 day post-exposure period, the study would have been prolonged until no symptoms were visible.
Because no case of mortality occurred slope and confidence interval could not be calculated using the Probit-Test (Toxcalc v 1.0). Summarizing of raw data as well as pre-calculations (mean and standard deviation) was performed by using "Excel 2002 for Windows©" of the Microsoft Corporation / USA.

RESULTS AND DISCUSSION

A. Findings

Acute oral toxicity to birds

Test substance	Spirotetramat formulation (BYI 08330 OD 150B G)
Test object	Chicken f
LD_{50} [mg formulation/kg bw]	> 2000
Lowest Lethal Dose (LDD) [mg formulation/kg bw]	> 2000

B. Observations

Mortality and behaviour
No mortality and no changes of behaviour occurred throughout the entire test period.

Body weight development
In all test groups including the control the body weight increased. At the end of the study the body weights of the 1000 mg formulation/kg bw - treatment group were slightly higher than in the control while at the 2000 mg formulation/kg bw – treatment group the body weight was slightly lower.

Food consumption
During the first three days after the exposure the food consumption in the treated group was lower than in the control. Afterwards all groups behaved similarly.

Gross pathology
Three birds of the 1000 mg formulation group and one bird of the 2000 mg formulation group showed an enlarged gall bladder.

These observations were not considered as pathological findings, but to be within natural variation, since there was no impact on physical condition or behaviour of the birds.

CONCLUSION

The LD_{50} for subadult chicken was higher than 2000 mg BYI 08330 OD150/kg bw. The lowest lethal dose (LLD) was > 2000 mg formulation/kg bw. No mortality or changes of behaviour occurred.

Report:	**KIIA 8.1.1/05, [redacted]; 2008c**
Title:	Acute oral toxicity for bobwhite quail (*Colinus virginianus*) with the test substance BYI 08330 OD 150B G Date: 2008-06-26
Organisation:	[redacted], Germany
Report No.:	BAR/LD 091; M-303530-01-1
Publication:	unpublished
Dates of experimental work:	April 21, 2008 – May 20, 2008
Guidelines:	US-EPA Pesticide Assessment Guidelines-Subdivision E, § 71-1 "Avian single-dose oral LD_{50} test" (October 1982), in consideration of US-EPA Ecological Effects Test Guideline "OPPTS 850.2100 Avian Acute Oral Toxicity Test" (April 1996). MAFF (UK)-Working Document No. 7/5c "Evaluating the acute oral and short-term cumulative oral toxicity of pesticides to birds"
Deviations:	No major deviations
GLP:	yes

Executive summary

The test item was administered as a single oral administration in gelatine capsules to individually housed adult bobwhite quail. 10 birds per treatment level (five males, five females) were exposed to 0 mg form./kg bw (control), 1000 mg form./kg bw and 2000 mg form./kg bw. The dosing was followed by a subsequent observation period of 14 days. The birds were observed on mortality, behavioural impacts and effects on food consumption and body weights. Additionally, gross pathological changes were determined by necropsies. Body weight was measured on day -1, day +7 and on day 14 (test termination).

No mortality occurred throughout the duration of the test.

Reduced vigilance was observed in individual birds on the application day in all test groups.

No impact on body weight could be determined.

Food consumption at the 2000 mg formulation/kg bw-treatment group was reduced between day 0 and day 3. No further differences between control and treatment group and no pathological changes at gross necropsy were observed.

The LD_{50} for adult bobwhite quail was higher than 2000 mg formulation/kg bw. The lowest lethal dose (LLD) was > 2000 mg formulation/kg bw.

MATERIAL AND METHODS

A Materials

1. Test material	**BYI 08330 OD 150B G**
Description	**Light brown suspension**
Lot/batch No.	**Batch-ID.: 2007-005113** **Tox No.: 07966-00**
Content of A.S.	**15.6% w/w (154.1 g/L)**
Density	**0.988 g/mL**

Stability of test compound	Approved until 2008-06-01 when stored at room conditions
2. Vehicle and/or positive control	Gelatine capsule, Size No.00 (one per bird) disintegration time:5 Minutes (US-federal standard No.285A-0,5% HCL)
3. Test animals	
Species	Bobwhite quail (*Colinus virginianus*)
Age	14 weeks old
Source	[redacted], Germany (hatched on 2007.11.23; date of supply : 2008.04.21)
Acclimation period	15 days under test conditions in the test facility
Environmental conditions	
Temperature	15 - 30°C
Photoperiod	14 h light / 10 h dark

B Study design and methods

1. In life dates: April 21, 2008 - May 20, 2008

2. Experimental treatments

Adult bobwhite quail (*Colinus virginianus*) were purchased from a commercial breeder. 10 birds per group (five males and five females) were allocated to each of the 2 treatment groups (1000 and 2000 mg a.s./kg bw) and one control group. All birds were identified by numbered and coloured leg bands.

Acclimation time lasted 15 days. One day prior to dosing, the body weights of the birds were determined for the calculation of the individual test-substance amounts. The birds were starved for 16 hours prior to oral administration of the test item. At all other times (acclimation time and post exposure period) the birds had free access to food.

The birds were fed with a standard rearing diet for quails (type: Alleinfutter für Wachteln, Batch 4317542 and 9848503, of the company "[redacted]; D[redacted]). Drinking water (Monheim City municipal water) was supplied *ad libitum* prior and throughout the study.

The birds were housed individually in stainless steel wired racks (38 x 50 cm x 23 cm) which were placed indoors in four rows – one upon the other. The front of each cage was equipped with a plastic-feeder.

3. Observations

Observations of mortality and signs of intoxication including regurgitation were made continuously during the first hour and approximately hourly on the day of dosing and at least once work-daily throughout the post exposure observation

period (body weights were recorded prior to the test initiation (day -1), on study (day +7) and on test termination (day +14).
On study days 3, and 7 (except day 14, only reweigh) all remaining food (per bird) was replaced by fresh food after cleaning. The removed food was reweighed for determination of the food consumption. At the end of the study all surviving birds were sacrificed by CO_2 asphyxiation.
Because no case of mortality occurred slope and confidence interval could not be calculated using the Probit-Test (Toxcalc v 2.0). Summarizing of raw data as well as pre-calculations (mean and standard deviation) was performed by using "Excel 2002 for Windows©" of the Microsoft Corporation / USA.

RESULTS AND DISCUSSION

A. Findings

Acute oral toxicity to birds

Test substance	Spirotetramat formulation (BYI08330 OD 150B G)
Test object	Bobwhite quail m/f
LD_{50} [mg formulation/kg bw]	> 2000
Lowest Lethal Dose (LDD) [mg formulation/kg bw]	> 2000

B. Observations

Mortality and behaviour
No mortality was observed. Signs of impairment of the digestive tract were seen in all test groups, mainly soft excrement, diarrhoea and excretion of uric acid. Since these findings occurred mainly on the day of dosing and also in the control they have to be considered more an effect of the dosing procedure than as test substance related.
Reduced vigilance and fluffed feathers were recorded in individual birds of all test groups mainly on the application day.

Body weight development
The body weights of the females were statistically higher than in the control, which is not considered to be treatment related. No further difference between test groups and treatment groups were found.

Food consumption
Food consumption was reduced at the 2000 mg formulation /kg body weight treatment group between Day 0 and Day 3. No further differences between control and treatment groups were identified.

Gross pathology

In the treatment groups, no pathological changes were found.

CONCLUSION

The LD_{50} for adult bobwhite quail was higher than 2000 mg BYI 08330 OD 150/kg bw. The lowest lethal dose (LLD) was > 2000 mg formulation/kg bw.

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

Report:	**KIIA 8.1.2/01, [redacted]; 2004**
Title:	BYI 08330 techn. a.s.: 5-Day-Dietary LC_{50} Bobwhite Quail (*Colinus virginianus*). Date: 2004-08-18
Organisation:	[redacted], Germany
Report No.:	BAR / LC017; M-084796-01-2
Publication:	Unpublished
Dates of experimental work:	October 9, 2003 - January 27, 2004 (Biological part)
Guidelines:	U.S.EPA Ecological Effects Test Guidelines, OPPTS 850.2200 Avian Dietary Toxicity Test (April 1996) OECD guideline 205 for testing of chemicals "Avian Dietary Toxicity Test" (April 1984)
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

Young Bobwhite quails (10-day-old chicks, 10 per diet group, two controls) were exposed for 5 days to nominal dietary concentrations of 313, 625, 1250, 2500 and 5000 mg a.s./kg feed corresponding to 300, 624, 1218, 2552 and 4997 mg measured a.s./kg feed. Exposure was followed by a subsequent 3-day observation period with untreated food.

The birds were observed on mortality, behavioural impacts and for effects on food consumption and body weight. Additionally, observations on gross pathological changes were performed by necropsies at study termination.

The LC_{50} for juvenile Bobwhite quails was found to be higher than 5000 mg a.s./kg feed.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330), tech.**
Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014 (mixed batch)** **Tox no.: TOX06344-00**
Purity	**97.5 %**
Stability of test compound	**Approved until 2003-12-23 when stored at room conditions**
2. Vehicle and/or positive control	**No carriers or solvents were used in the treated feed or the control feed (rearing diet for quails, type: "Wachtel und Entenhaltungsfutter (Quail and Mallard food), batch No. 50 / 03 of the company "Kliba GmbH")**
3. Test animals	
Species	**Bobwhite quail (*Colinus virginianus*)**
Age	**10 days**

Source	[redacted]
	Germany
Acclimation period	**Three days prior to test initiation 70 uniformly developed birds were selected for randomized assignment to the exposure groups and controls.**
Environmental conditions	
Temperature	**18.6 - 21.1°C**
Photoperiod	**10 hrs light / 14 hrs dark**

B Study design and methods

1. In life dates **August 19, 2003 - September 03, 2003**

2. Experimental treatments

Three days prior to exposure the chicks were allocated randomly to each of the five treatment levels and two control groups. Each group consisted of 10 chicks. Afterwards, the test units were arranged in a randomized order.
The birds were individually identified by leg bands and were observed daily on health and compatibility until start of exposure.
At all times, birds had free access to food (rearing diet for quails, type: "Wachtel und Entenhaltungsfutter) and fresh tap water (Monheim City municipal water).
During the post exposure period, weighed amounts of untreated food were placed into the cage-feeders. In daily intervals, all uneaten food was removed from the feeders and feeders were refilled with fresh food after cleaning. During exposure and post-exposure period, the remaining uneaten food / diet was re-weighed for determination of food consumption. After weighing, the uneaten amounts were disposed.
The a.s. content, stability and homogeneity data of the diet were elaborated by using food batches (prepared October 21, 2003) and obtained from the department for toxicological analysis of [redacted], Germany (analytical report included in the current study report).
Observations on signs of intoxication were made daily during acclimation, twice on the first exposure day, continued at least once daily throughout the following study days until terminal sacrifice.
At the end of the study the birds were sacrificed by CO_2 asphyxiation and gross necropsies were carried out on all survivors.
Body weights were determined at the beginning of exposure (day 0), at the end of exposure (day 5) and after terminal sacrifice (day 8).

3. Observations

- **Individual body weight development, based on the body weight at the start of exposure (day 0), day 5 and at study termination (day 8)**
- **Body weight of all premature deaths**
- **Daily food consumption per group for the periods day 0 - 4, and day 5 - 7, calculation of the daily food intake/bird and of the daily dose/bird**
- **Mortality**

- **Sublethal effects**
- **Results of gross necropsy of all prematurely deaths and of all survivors after sacrifice**

RESULTS AND DISCUSSION

A. Findings

The test material content in the animal rations agreed with the target concentrations within the requested limits of 20% deviation from the nominal values (measured concentrations between 96 and 102% of nominal).

5-Day-Dietary toxicity to birds

Test substance	Spirotetramat techn.
Test object	Bobwhite quail
LC_{50} [mg a.s./kg food]	> 5000
Lethal Daily Dietary Dose [mg a.s./kg bw/d]	> 498
Highest tested concentration without effect (NOEC) [mg a.s./kg feed]	2500

B. Observations

Mortality and Behaviour: One chick out of the 1250 mg a.s./kg feed group was sacrified prematurely because of an injury. The cloaca of this chick was pecked by the other chicks and therefore agglutinated with blood and excrement. During the exposure period the 5000 mg a.s./kg feed - group showed signs of intoxication like soft excrement. These symptoms ceased completely one day after exposure. No behavioural impacts were observed at any treatment group.

Body Weight Development: No differences between exposure groups and control were found.

Food Consumption: The chicks of the 5000 mg a.s./kg feed group and the 2500 mg a.s./kg feed group avoided the treated food. They consumed 30 - 50% less of the amount of the control groups during the exposure period. By this way the dose ingested was respectively lower as expected, when food consumption is predicted on the base of the feeding rate of the control.
The other dosed groups showed no difference in food consumption. During the post exposure period no difference between exposure groups and control were found.

Gross Necropsy Results: Birds anatomy did not display any visible signs of toxic effects from the active ingredient in the post-mortem examination of the birds from any group.

CONCLUSION

The LC_{50} for juvenile Bobwhite quails was higher than 5000 mg a.s./kg feed. This corresponds to a Lethal Daily Dietary Dose of > 498 mg a.s./kg bw/d. The NOEC was determined as 2500 mg a.s./kg feed.

IIA 8.1.3 Avian dietary toxicity (5-day) test in a second unrelated species

Report:	**KIIA 8.1.3/01, [redacted] 2005**
Title:	Technical BYI 08330: A Subacute Dietary LC_{50} with Mallards. Date: 2006-05-03
Organisation:	Bayer CropScience, Ecotoxicology, South Metcalf, Stilwell, Kansas
Report No.:	EBFNX012; M-274385-01-1
Publication:	Unpublished
Dates of experimental work:	March 28, 2005 – April 8, 2005
Guidelines:	EPA 71-2, OPPTS 850.2200, OECD Guideline No. 205
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

Young Mallard ducks (10-day-old hatchlings, 10 hatchlings per test level) were exposed for 5 days to nominal dietary concentrations of 0 (control), 312, 625, 1250, 2500 and 5000 mg a.s./kg feed. The exposure was followed by a subsequent 3-day observation period on untreated feed. The measured concentrations in the diet were 0 (<8.75), 344, 708, 1436, 2843, and 6050 mg a.s./kg feed and the mean daily dietary dose was 0, 65, 120, 233, 384, and 475 mg a.s./kg body weight/day, respectively. Mortality, clinical symptoms, body weight, and feed consumption were monitored. Post-mortem examinations were conducted on a percentage of the birds at study termination.

The dietary LC_{50} of technical BYI 08330 in Mallard ducks was determined to be greater than 6050 mg a.s./kg feed.

MATERIAL AND METHODS

A Materials

1. Test material

Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014**
Purity	**97.1%**
Stability of test compound	**2005-05-05, stable under ambient (25 ± 5°C) storage conditions**

2. Vehicle and/or positive control

No carriers or solvents were used in the treated feed or the control feed ([redacted] Bayer Starter Ration, Batch no. 13-07041)

3. Test animals

Species	**Mallard ducks (*Anas platyrhynchos)***
Age	**10 days**
Source	[redacted]
Acclimation period	**5 days prior to being randomized into test groups**

Environmental conditions

Temperature	**Room temperature approx. 22°C/brooding compartment of each cage approx. 32°C to 38°C**
Relative humidity	**Approx. 56% during acclimation and test periods**
Photoperiod	**14/10 h light/dark cycle with a 30 minute dawn/dusk cycle (wide spectrum illumination)**

B Study design and methods

1. In life dates **March 28, 2005 – April 8, 2005**

2. Experimental treatments

Two-day old mallard ducklings were received from [redacted], [redacted] on March 23, 2005. The ducklings were housed in galvanized steel brooders (91L x 81W x 25H cm). Room lighting (wide spectrum) was maintained at a 14/10 h light/dark cycle with a 30 minute dawn/dusk cycle. Food ([redacted] Bayer Starter Ration) and water (local tap water) were available *ad libitum* prior to and during the study.

Birds were acclimatised for five days prior to being randomized into test groups. Only birds that appeared healthy were used for the study. All birds were fed basal diet for the acclimation and pre-exposure periods.

On day -3, approximately 140 birds were weighed and 60 of these were randomized into study cages, with 10 birds per cage. The study consisted of six brooder cages, five for treatment levels and one for a control level. Once test assignments were complete, feed consumption was monitored for three days prior to test initiation. Following the three-day pre-exposure period, diets treated with technical BYI 08330 were administered to treatment level birds. The birds were approximately 10 days old when the treated diets were administered. Control birds received basal (untreated) feed. After five days of exposure, treatment level birds were given a control diet for a three-day recovery period. During the pre-exposure, exposure, and recovery periods, feed pans were emptied each day and fresh feed was added. Birds that survived to study termination were sacrificed by CO_2 asphyxiation. All surviving birds at the high level were necropsied. No treatment related gross lesions or unusual observations were found at the high level, therefore, only 40% of the birds in the lower treatment levels were examined.

3. Observations

Body weights were recorded on day -3, at study initiation (day 0), after five days of exposure (day 5), and study termination (day 8). Feed consumption for each level was recorded daily during the pre-exposure, exposure, and recovery periods.

Observations for mortality and clinical signs of toxicity were recorded approximately one, two, and four hours after Day 0 treated diet administration and twice daily throughout the remainder of the study except on weekends, holidays and day 8 (study termination) when only one observation per day was made. Bedding was changed at least weekly.

Body weight and growth data were subjected to statistical analysis. Normality and homogeneity of variance of the data were tested using the chi-square test (α = 0.01) and the Levene's test (α = 0.05), respectively. The statistical analyses were conducted using TOXSTAT, version 3.4 software (WEST, Inc. and Gulley, 1994).

RESULTS AND DISCUSSION

A. Findings

The nominal BYI 08330 technical concentrations were 0 (control), 313, 625, 1250, 2500 and 5000 mg a.s./kg feed. The measured concentrations were 0 (<8.75), 344, 708, 1436, 2843, and 6050 mg a.s./kg feed representing a range of 110 to 121% of nominal. All subsequent designations of treatment concentrations will refer to the measured concentrations.
No LC_{50} (median lethal concentration) could be calculated as no treatment related mortalities occurred at any treatment level.

5-Day-Dietary toxicity to Mallard duck

Test substance	Spirotetramat techn.
Test object	Mallard duck
Exposure	5 day dietary
LC_{50} [mg a.s./kg feed]	> 6050
Lethal Daily Dietary Dose [mg a.s./kg bw/d]	> 475
Lowest lethal concentration (LLC) [mg a.s./kg feed]	> 6050
Lowest observed adverse effect level (LOAEL) [mg a.s./kg feed]	708
Highest tested concentration without effect (NOAEC) [mg a.s./kg feed]	344

B. Observations

Mortality and Behaviour: No mortalities occurred during the study and no symptoms of toxicity were noted in any birds during the study

Body Weight Development: For all body weight parameters, the data were normally distributed (chi square test, $p > 0.01$) and variances were homogenous (Levene's test, $p > 0.05$), indicating parametric procedures to be appropriate.
Body weights for Day 0 resulted in no statistical difference among treatments relative to the control.
Body weights for Day 5 and body weight change from Day 0 to Day 5 exhibited a statistical difference at all treatment levels relative to the control. The NOAEL based on body weight over the 5-day exposure period of the study was < 344 mg a.s./kg feed and the LOAEL was 344 mg a.s./kg feed.
Body weight change from Day 5 to Day 8 exhibited a statistical difference at all treatment levels relative to the control. However, these were not an adverse effect since the increase in bodyweight at the treatment levels was greater than in the control in all cases. The ducklings were gaining weight during the recovery period.
Body weights on Day 8 and body weight change from Day 0 to Day 8 were analysed. There were significant differences from the controls at 708, 1436, 2843, and 6050 mg a.s./kg feed treatment levels. Therefore, taking into consideration the exposure and recovery phases of the study, the NOAEL was 344 mg a.s./kg feed and the LOAEL was 708 mg a.s./kg feed.

Food Consumption: Due to the fact that there were no replicates for feed consumption (based on guideline study design) no statistical analysis could be performed on this parameter.

There was a reduction in feed consumption in all of the treatment levels during the exposure period as compared to the control group. The biological NOAEL based on mean feed consumption over the 5-day exposure period was < 344 mg a.s./kg feed and the LOAEL was 344 mg a.s./kg feed.
During the 3 day recovery period of the study, the 344, 708, 1436, and 2843 mg a.s./kg feed treatment levels had a significant increase in food consumption and a return to normal levels or higher levels of consumption as compared to the controls. There was still a reduction in feed consumption at the 6050 mg a.s./kg feed treatment level as compared to the control group. Therefore, taking into consideration the exposure and recovery phases of the study, the biological NOAEL was 2843 mg a.s./kg feed and the LOAEL was 6050 mg a.s./kg feed. The daily dietary doses were 0, 65, 120, 233, 384, and 475 mg a.s./kg bw/day for the control, 344, 708, 1436, 2843, and 6050 mg a.s./kg feed treatment levels, respectively.

Gross Necropsy Results: Postmortem examinations revealed no treatment related gross lesions or unusual observations

CONCLUSION

The dietary LC_{50} of technical spirotetramat in Mallard duck was determined to be > 6050 mg a.s./kg feed, equivalent to 475 mg a.s./kg bw/day, as no treatment related mortalities occurred at any treatment level. Based on all parameters measured, the NOAEL was 344 mg a.s./kg feed and the LOAEL was 708 mg a.s./kg feed. Body weight and growth were the most sensitive sub-lethal endpoints.

IIA 8.1.4 Subchronic and reproductive toxicity to birds

Report:	**KIIA 8.1.4/01, [redacted] and [redacted].; 2006**
Title:	Effect of Technical BYI 08330 on Northern Bobwhite Reproduction
	Date: 2006-09-21
Organisation:	[redacted], Germany
Report No.:	EBFN0220; M-277757-01-1
Publication:	Unpublished
Dates of experimental work:	June 14, 2004-January 17, 2005
Guidelines:	USEPA FIFRA 71-4
	OPPTS 850.2300 (Draft)
	OECD 205
	ASTM standard E 1062-86
Deviations:	None
GLP:	yes

Executive summary

This study is a one-generation reproduction study design with the purpose to evaluate the effects of dietary exposure to technical BYI 08330 on the health and reproductive capacity of

adult northern bobwhites (*Colinus viginianus*) as a surrogate species. Effects on adult health, weight gain, feed consumption, and reproductive parameters were monitored and evaluated and expressed as NOEC and LOEC.

Adult northern bobwhite were exposed to nominal dose levels of BYI 08330 technical grade active ingredient at dose levels of 0 (control), 80, 240, and 720 mg a.s./kg feed for 11 weeks prior to egg-laying and for 11 weeks during the reproductive phase of the study. Body weight, feed consumption, clinical appearance, reproductive parameters, mortality, and post mortem findings of the parental generation were monitored. Reproductive parameters included egg production, egg fertility, embryo survival, hatchability, hatchling body weight, 14-day survival, 14-day survivor body weight, and eggshell strength/thickness.

The reported results refer to the mean measured concentrations of BYI 08330 in feed (mg a.s./kg feed). The spirotetramat NOEC for adult health was 264 mg a.s./kg feed (daily dietary NOEC 23 mg a.s./kg bw/day) based on a 14% reduction in female body weight gain at 802 mg a.s./kg feed. For the reproductive endpoints, a statistically significant effect was noted for hatchling body weight at 802 mg a.s./kg feed. This parameter was reduced by approximately 5% as compared to the control, but was no longer significantly reduced in the 14-day hatchlings. Because of the small reduction and the lack of effect in the 14-day hatchling weights, this effect was not considered biologically relevant. Therefore, the NOEC for the reproductive parameters was the highest dose tested of 802 mg a.s./kg feed (daily dietary NOEC of 74 mg a.s./kg bw/day).

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014**
Purity	**97.2% (different from same batch in other summary)**
Stability of test compound	**Expiration date: 2005-05-05 when stored at room temperature**
2. Vehicle and/or positive control	**None**
3. Test animals	
Species	**Northern bobwhite (*Colinus viginianus*)**
Age	**16 weeks of age; approaching first breeding season**
Source	[redacted]
Acclimation period	**23 days**
Replicates	**18 breeding pairs per dose level; one male and one female per pen**
Environmental conditions	

Temperature	**Adults – 22°C and 56% relative humidity (on average)** **Hatchlings – 32-38°C in the brooding compartments with a room temperature of 22°C and 56% relative humidity (on average)**
Photoperiod	**Adults** **Acclimation and pre-reproductive phase - 7 hours light/17 hours dark with a 30 minutes transition period** **Reproductive phase - 17 hours light/7 hours dark with a 30 minutes transition period** **Light intensity was between 3.0-11.3 foot candles** **Hatchlings** **14 hours light/10 hours dark** **Wide spectrum illumination**

B Study design and methods

1. In life dates **June 14, 2004-January 17, 2005**

2. Experimental treatments

Adult birds were impartially placed in cages as pairs upon arrival. The pairs were randomly assigned to a treatment level by cage. The mean body weight and standard deviation for all adults of each sex was determined. All birds used in the study were in an acceptable body weight range (mean ± twice the standard deviation).

Three of the four groups were administered treated diets containing nominal concentrations of 80, 240, or 720 mg a.s./kg feed during the study. The fourth group, designated as the control group, was administered raw feed. The offspring produced during the study were housed according to parental treatment group and hatch day. All hatchlings received an untreated starter diet. All birds are provided food and water *ad libitum* throughout the study.

The adult diet used for all phases of the study was [redacted] Bayer Game Bird ration. The compound was mechanically mixed into the adult feed without carriers or solvents.

Mixed adult feed was stored at approximately -10ºC. Feed mixing was performed approximately once per week. The feed mixing procedure was confirmed by an analysis of the diet homogeneity. The stability of BYI 08330 in avian diet under freezer and room conditions was also determined.

The hatchling feed during the study was [redacted] Bayer Starter Ration. Since the hatchlings received untreated feed, hatchling feed was not mechanically mixed.

The measured concentration of BYI 08330 was determined in the treated diet. Samples taken on weeks 1, 5, 10, 15 and 20 of the dosing phase of the study were analysed to measure the BYI 08330 technical concentration in the diet.

For statistical analysis data from treatment groups were compared to controls using the Shapiro-Wilk's test for normality and Levene's test of equal variance to determine if dose groups had unequal variances. If normality and variances were equal ($p < 0.05$), then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test or William's test. If variances were unequal, then the nonparametric analyses were conducted using the Jonckheere or Mann-Whitney procedures. Statistical significance is reported at $p<0.05$.

3. Observations

Adult body weights were measured at study initiation (week 1), every other week until the egg production phase (week 3, 5, 7 and 9), and on the day the adult birds were sacrificed. No adult body weights were taken during the egg production phase. Adult feed consumption was measured weekly by cage throughout the study. Adult birds were observed at least once daily for signs of toxicity, injuries, illness, or mortality. At terminal adult sacrifice, the birds were sacrificed by carbon dioxide gas asphyxiation, weighed and necropsied.

The following parameters were statistically analysed.

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

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

Eggs were collected twice daily (once on weekends and holidays) and labelled according to parental cage and date. Eggs were placed on a rack, covered with a plastic bag, and stored in an Omnicube egg cooler. Egg cooler temperature averaged approximately 59ºF (15ºC) and relative humidity averaged approximately 55%. Any eggs that were not able to be set (too small, too soft, obviously broken) were recorded and discarded at collection or prior to egg set. To prevent pathogen contamination, eggs were fumigated with formaldehyde gas in a sealed chamber using a CERTEK Formaldehyde Generator/Neutralizer prior to setting in the incubator.

Eggs were removed from the egg cooler weekly and candled to detect shell cracks. Cracked eggs were recorded and discarded. Eggs were placed into a NatureForm Incubator. The transfer of the eggs to the incubator was referred to as "egg set". Incubator temperature averaged approximately 99ºF (37ºC) with an average relative humidity of approximately 61%. In order to prevent adhesion of the embryo to the shell membrane, the incubator was equipped with an automatic egg rotation device designed to rotate the eggs approximately every two hours. Eggs were candled again on day 11 of incubation to determine embryo viability (fertility) and on day 18 to determine embryo survival. Nonfertile and nonviable eggs were removed, recorded, and discarded.

On day 21 of incubation, the eggs were placed into a NatureForm Hatcher and allowed to hatch. The temperature and humidity are monitored at one point in the hatcher. Minor differences in temperature and humidity from the set point are to

be expected in a properly functioning hatcher. Hatcher temperature averaged approximately 99ºF (37ºC) with an average relative humidity of approximately 76%. The hatching trays were compartmentalised in order to keep hatchlings separated by parental cage.
Hatchlings were removed from the hatching compartments on days 24 and 25 of incubation. They were wing banded and weighed. The hatchlings were group housed in brooders according to week of hatch and parental treatment group. Wing bands made it possible to identify hatchlings to a specific parental cage. The unhatched eggs per parental cage were recorded and discarded.
All eggs laid on one day every other week during the egg laying period were collected for eggshell strength and thickness measurements. Eggshell strength measurements were taken at one point on the waist using a Chatillon Model DFG, Digital Force Gauge calibrated to 0.01 kg units. Following eggshell strength measurements, eggs were opened at the waist, contents removed, and the eggshells thoroughly washed. Eggshells were allowed to air dry for a minimum of 48 hours at room temperature. The average thickness of the dried shell plus the membrane was determined by measuring three points around the waist of the egg using a Mitutoyo Spherical Anvil micrometer. Measurements were made to the nearest 0.001 mm.

The following parameters were statistically analysed.
Eggs Laid (mean): Analysed as the total egg production. Eggs laid will be analysed on a per hen basis.
Eggs Cracked (mean): Cracked eggs are determined by candling prior to incubation. Cracked eggs will be analysed on a per hen basis.
Eggs Set (mean): Eggs placed under incubation are "set." Analysed as the total number during the dosing phase. Eggs set will be analysed on a per hen basis.
Viable Embryos (mean): Viability (fertility) is determined by candling on the 11th day of incubation. Viability (fertility) analysed as total viable embryos. Viable embryos were analysed on a per hen basis.
Live Embryos (mean): Live embryos are determined by candling on the 18th day of incubation. Analysed as the total number of live embryos on a per hen basis.
Eggs Not Cracked of Laid (%): Eggs laid that were not cracked as a percentage of eggs laid on a per hen basis. Analysed as the mean percentage during the reproductive phase.
Eggs Set of Laid (%): Eggs set as a percentage of eggs laid on a per hen basis. Analysed as the mean percentage during the reproductive phase.
Viable Embryos of Eggs Set (%): Viable embryos as a percentage of eggs set on a per hen basis. Analysed as the mean percentage during the reproductive phase.
Live Embryos of Viable Embryos (%): Live embryos as a percentage of viable embryos on a per hen basis. Analysed as the mean percentage during the reproductive phase.
Eggshell Strength: The force needed to penetrate the shell and membrane measured at one point on the waist of the egg, analysed on a per hen basis.

Eggshell Thickness: The thickness of the shell plus the membrane measured at three points around the waist of the egg, analysed on a per hen basis.
Hatchling body weights were measured and recorded on the day they were removed from the hatcher and on day 14. Feed consumption for the offspring was not monitored. Hatchlings were observed once daily throughout the 14-day period for signs of toxicity, injuries, illness, and mortality. A record was maintained of all observations. Mortalities that occurred prior to the end of the 14-day period were recorded. Hatchlings that survived the 14-day observation period were sacrificed by CO_2 asphyxiation and weighed.

The following parameters were statistically analysed.
Number Hatched (mean): Live hatchlings that had liberated themselves from their eggs by day 25 of incubation. Analysed as the total number of normal hatchlings on a per hen basis.
14-day-old Survivors (mean): Live chicks at 14 days post hatch. Analysed as the total number of 14-day survivors on a per hen basis.
Number Hatched of Eggs Laid (%): Hatchlings as a percentage of eggs laid on a per hen basis. Analysed as the mean during the reproductive phase.
Number Hatched of Eggs Set (%): Hatchlings as a percentage of eggs set on a per hen basis. Analysed as the mean during the reproductive phase.
Number Hatched of Live Embryos (%): Hatchlings as a percentage of live embryos on a per hen basis. Analysed as the mean during the reproductive phase.
14-day-old Survivors of Eggs Set (%): Live chicks at 14-days post hatch as a percentage of eggs set on a per hen basis. Analysed as the mean during the reproductive phase.
14-day-old Survivors of Number Hatched (%): Live chicks at 14-days post hatch as a percentage of hatchlings on a per hen basis. Analysed as the mean during the reproductive phase.
Hatchling Body Weight: Individual weights of the live hatchlings taken upon removal from the hatcher, analysed on a per hen basis. Analysed as the overall mean during the reproductive phase.
14-day-old Survivor Body Weight: Individual weights of the 14-day-old offspring taken at sacrifice, analysed on a per hen basis. Analysed as the overall mean during the reproductive phase.

RESULTS AND DISCUSSION

A. Findings

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

The analytical findings (in feed by HPLC-UV, limit of Quantitation (LOQ) = 8.75 mg a.s./kg feed) reflect the expected nominal concentrations of the test item. The mean measured concentrations of spirotetramat were 85, 264, 802 mg a.s./kg feed. The control feed measured <8.75 mg a.s./kg feed in all cases. The reported results refer to the mean measured

concentrations of BYI 08330 in feed. The results can also be expressed as a calculated daily dose based on estimated food consumption corresponding to 0, 8, 23, and 74 mg a.s./kg bw/day. All subsequent observations will refer to the mean measured concentrations in mg a.s./kg feed (ppm).

Seven adult birds died during the exposure period; two control hens, two hens at the 85 mg a.s./kg treatment level and one hen at the 802 mg a.s./kg treatment level. There was also one male bird that died during the study in the 85 mg a.s./kg treatment level and one male bird in the 802 mg a.s./kg treatment level. There was no significant difference in adult mortality as compared to the control at any treatment level. The NOEC was 802 mg a.s./kg and the LOEC was greater than 802 mg a.s./kg.

Postmortem Examination Results for Bobwhites Fed BYI 08330 in the Diet

		Measured Dietary Concentration (mg a.s./kg feed)			
Sex	Observation at Necropsy	Control	85	264	802
		(Number with Observation/Number Examined)			
Male	Found Dead	0/18	1/18	1/18	0/18
	Sacrificed	2/18	2/18	2/18	0/18
	Feather Loss	2/16	0/4	1/4	2/18
Female	Found Dead	2/18	2/18	2/18	0/18
	Sacrificed	0/18	1/18	1/18	0/18
	Feather Loss	9/16	1/4	1/4	7/18
	Gross Lesions	1/16	1/4	0/4	3/18
	Skin Lesions/Abrasions	1/16	1/4	0/4	3/18
	Regressed Ovaries	1/16	1/4	0/4	1/18
	Post Mortem Autolytic Signs	0/16	0/4	1/4	2/18

Statistical Results by Test Parameter

Parameter	Control	85 ppm	264 ppm	802 ppm	NOEC/ LOEC
Eggs laid/pen	48.17	43.61	44.72	54	802 ppm >802 ppm
Eggs cracked/pen	0.11	0.67	0.06	0.5	802 ppm >802 ppm
Eggs not cracked/eggs laid (%)	99.56	97.83	99.9	98.92	802 ppm >802 ppm

Eggs set/pen	44.61	39.17	40.83	48.94	802 ppm >802 ppm
Shell thickness	0.21	0.20	0.20	0.20	802 ppm >802 ppm
Eggshell strength	0.75	0.72	0.80	0.87	802 ppm >802 ppm
Eggs set/eggs laid (%)	93.04	89.75 [a]	86.4 [b]	90.38	802 ppm >802 ppm
Viable embryo/pen	42.33	37.44	38.72	45.72	802 ppm >802 ppm
Viable embryos/eggs set (%)	95.59	94.99	94.44	93.13 [d]	802 ppm >802 ppm
Live embryos/pen	42.11	37.11	38.50	45.17	802 ppm >802 ppm
Live embryo/viable embryo (%)	99.43	98.93	99.57	98.94	802 ppm >802 ppm
No. of hatchlings/pen	40.11	34.61	35.06	42.28	802 ppm >802 ppm
No. of hatchlings/eggs laid (%)	81.72	79.26	73.11	78.26	802 ppm >802 ppm
No. of hatchlings/eggs set (%)	88.01	87.97	84.77	86.54	802 ppm >802 ppm
No. of hatchlings/live embryo (%)	92.73	93.03	89.57	93.64	802 ppm >802 ppm
Hatchling survival/pen	42.24	40.93	36.76	42.17	802 ppm >802 ppm
Hatchling survival/eggs set (%)	87.46	86.87	83.72	86.35	802 ppm >802 ppm
Hatchling survival/no. of hatchlings (%)	99.43	98.80	98.07	99.80	802 ppm >802 ppm
Hatchling weight (g)	7.2	6.9	7.0	6.8	802 ppm >802 ppm
Survivor weight (g)	35.5	35.6	35.5	34.5	802 ppm >802 ppm
Mean food consumption (g/bird/day)	20.6	20.2	19.3	19.5	802 ppm >802 ppm
Male weight gain (g)	28	27	23	25	802 ppm >802 ppm
Female weight gain (g)	62	54	57	49 [e]	264 ppm 802 ppm

[a] Statistically significant per Mann-Whitney analysis, but not biologically relevant because the reduction is only 1.85% when the median is compared to the control and there is no dose-

response relationship.
[b] Statistically significant per Mann-Whitney analysis, but not biologically relevant because the reduction is 0.7% when the median is compared to the control and there is no dose-response relationship.
[c] Statistically significant per Jonckheere's analysis, but not appropriate for these data because of the lack of a dose-response relationship.
[d] Statistically significant per Jonckheere's analysis. However, the reduction is only 3% as compared to the control and is not considered biologically significant.
[e] Statistically significant per Jonckheere's test (14% reduction compared to control).

B. Observations

No overt signs of intoxication were observed during the study in any adult test group. Occurrences of feather loss, abrasions (small lacerations), etc., all associated with normal laboratory cage housing, were observed in the control and all treatment levels. There were no significant clinical symptoms or compound related effects observed during the study. There were no compound related lesions found during the postmortem examinations. The NOEC was 802 mg a.s./kg and the LOEC was greater than 802 mg a.s./kg.

The offspring were observed for clinical symptoms during the 14-day growth period. There were no compound-related deaths or abnormal signs. The NOEC was 802 mg a.s./kg and the LOEC was greater than 802 mg a.s./kg.

CONCLUSION

Based on all parameters, and the consideration of biological relevance, the lowest-observed-effect concentration (LOEC) for adult northern bobwhites exposed to technical BYI 08330 in the diet was 802 mg a.s./kg feed or 74 mg a.s./kg bw/day and the NOEC was 264 mg a.s./kg feed or 23 mg a.s./kg bw/day for the adult growth observations. The LOEC for the reproductive parameters was greater than 802 mg a.s./kg feed or greater than 74 mg a.s./kg bw/day. The NOEC for the reproductive parameters was considered to be 802 mg a.s./kg feed or 74 mg a.s./kg bw/day.

Report:	**KIIA 8.1.4/02, [redacted] and [redacted] 2006**
Title:	Effect of Technical BYI 08330 on Mallard Reproduction.
	Date: 2006-09-22
Organisation:	[redacted], Germany
Report No.:	EBFNX013, M-277794-01-1
Publication:	Unpublished
Dates of experimental work:	March 1, 2005-July 26, 2006
Guidelines:	USEPA FIFRA 71-4
	OPPTS 850.2300 (Draft)
	OECD 206
	ASTM standard E 1062-86
Deviations:	None

GLP: yes

Executive summary

This study is a one-generation reproduction study design with the purpose to evaluate the effects of dietary exposure to technical BYI 08330 on the health and reproductive capacity of adult mallard ducks (*Anas platyrhynchos*) as a surrogate species. Effects on adult health, weight gain, feed consumption, and reproductive parameters were monitored and evaluated and expressed as NOEC and LOEC.

Adult mallards were exposed to nominal dose levels of BYI 08330 technical grade active ingredient at dose levels of 0 (control), 80, 240, and 720 mg a.s./kg feed (nominal) for 11 weeks prior to egg-laying and for 10 weeks during the reproductive phase of the study. Body weight, feed consumption, clinical appearance, reproductive parameters, mortality, and post-mortem findings of the parental generation were monitored. Reproductive parameters included egg production, egg fertility, embryo survival, hatchability, hatchling body weight, 14-day survival, 14-day survivor body weight, and eggshell strength/thickness.

The reported results refer to the mean measured concentrations of BYI 08330 in feed (89, 269, and 869 mg a.s./kg feed).

Adult parameters: A statistically significant adverse effect was determined at the 269 mg a.s./kg treatment level for male and female weight gain and food consumption. The NOAEC was 89 mg a.s./kg feed.

Egg parameters: A statistically significant adverse effect was determined at the 269 mg a.s./kg treatment level for eggs laid, eggs set, viable embryos, live embryos, number hatched, percent live embryos of viable, number hatched of laid, number hatched of eggs set and number hatched of live embryos. The NOAEC was 89 mg a.s./kg feed.

Hatchling parameters: A statistically significant adverse effect was determined at the 269 mg a.s./kg treatment level for the number of 14-day survivors, survivor weight, 14-day survivors of eggs set and 14-day survivors of number hatched. A statistically significant difference was determined at the 89 mg a.s./kg treatment level for hatchling body weight. However, since the difference in body weight at time of hatch between the 89 mg a.s./kg treatment level and controls was small (6.6%) and no similar statistical difference was noted at day 14 post-hatch, this finding was not considered biologically adverse. Therefore, the NOAEC was 89 mg a.s./kg feed.

Clinical Observations and Gross Pathology: Cracking and peeling of the mallard feet were observed in the 89, 269 and 869 mg a.s./kg treatment levels. Foot lesions at the 269 and especially 869 mg a.s./kg treatment levels were in some cases severe enough to impair normal standing and feeding behaviour. The foot symptoms at the 89 mg a.s./kg treatment level were

less severe and did not affect behaviour. There were no other apparent compound related findings. The NOAEC was 89 mg a.s./kg feed. The NOEC was less than 89 mg a.s./kg feed.

Conclusions: Although there were foot effects and a statistically significant reduction in hatchling body weight at the 89 mg a.s./kg feed level, these effects were not considered adverse or biologically significant. After a review of all parameters, the NOAEC for adult mallard exposed to technical BYI 08330 in the diet was 89 mg a.s./kg feed or 9 mg a.s./kg bw/day. The LOAEC was 269 mg a.s./kg feed or 22 mg a.s./kg bw/day based on effects on multiple parental and reproductive endpoints including adult body weight gain and food consumption, egg production, hatching success, hatchling weight and number of 14-day survivors.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014**
Purity	**97.1%**
Stability of test compound	**Expiration date: 2005-05-05 when stored at room temperature. Reanalysed April 14, 2005 with a.s. = 97.99%**
2. Vehicle and/or positive control	**None**
3. Test animals	
Species	**Adult mallard ducks (*Anas platyrhynchos*)**
Age	**14 weeks of age; approaching first breeding season**
Source	**[redacted]**
Acclimation period	**3 weeks**
Replicates	**15 breeding pairs per dose level; one male and one female per pen**
Environmental conditions	
Temperature	**Adults – 71ºF (22ºC) and relative humidity approximately 55% (on average)** **Hatchlings – 90ºF - 100ºF (32ºC - 38ºC) in the brooding compartments with a room temperature of 72ºF (22ºC) and relative humidity approximately 56% (on average)**
Photoperiod	**Adults** **Acclimation and pre-reproductive phase - 7 hours light/17 hours dark with a 30 minutes transition period**

Reproductive phase - 17 hours light/7 hours dark with a 30 minutes transition period
Light intensity was between 3.8 to 11.2 foot candles
Hatchlings
14 hours light/10 hours dark
Wide spectrum illumination, 14.6 foot candles

B Study design and methods

1. In life dates March 1, 2005- July 26, 2006

2. Experimental treatments

Adult birds were impartially placed in cages as pairs upon arrival. The pairs were randomly assigned to a treatment level by cage. The mean body weight and standard deviation for all adults of each sex was determined. All birds used in the study were in an acceptable body weight range (mean ± twice the standard deviation).
Three of the four groups were administered treated diets containing nominal concentrations of 80, 240, or 720 mg a.s./kg feed during the study. The fourth group, designated as the control group, was administered raw feed. The offspring produced during the study were housed according to parental treatment group and hatch day. All hatchlings received an untreated starter diet. All birds are provided food and water *ad libitum* throughout the study.
Each adult cage was identified with a color-coded (by treatment) label containing the study number, cage number, dietary level, and bird band numbers. Wing bands having an individual animal number identified adult birds. All eggs laid during the study were labeled in pencil with the cage number and date. Leg bands having an individual number for that hatch identified hatchlings. Labels containing the study number, parental dietary concentration, and hatch date identified the brooders used for the offspring.
The adult diet used for all phases of the study was [redacted] Bayer Game Bird ration. The compound was mechanically mixed into the adult feed without carriers or solvents.
Mixed adult feed was stored at approximately -10ºC. Feed mixing was performed approximately once per week. Historical data confirmed BYI 08330 diet homogeneity, freezer and room stability (Effect of Technical BYI 08330 on Northern Bobwhite Reproduction, [redacted], T.J., 2006, unpublished report).
The hatchling feed during the study was [redacted] Bayer Starter Ration. Since the hatchlings received untreated feed, hatchling feed was not mechanically mixed.
The measured concentration of BYI 08330 was determined in the treated diet. Samples of both control and treated adult diets were taken weekly, immediately after mixing, and frozen. Samples taken on weeks 1, 5, 10, 15 and 20 of the dosing phase of the study were analysed to measure the BYI 08330 technical concentration in the diet.
For statistical analysis data from treatment groups were compared to controls using the Shapiro-Wilk's test for normality and Levene's test of equal variance to determine

if dose groups had unequal variances. If normality and variances were equal ($p < 0.05$), then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test or William's test. If variances were unequal, then the nonparametric analyses were conducted using the Jonckheere or Mann-Whitney procedures. Statistical significance is reported at $p<0.05$.

3. Observations

Adult body weights were measured at study initiation (week 1), every other week until the egg production phase (week 3, 5, 7 and 9), and on the day the adult birds were sacrificed. No adult body weights were taken during the egg production phase. Adult feed consumption was measured weekly by cage throughout the study.

Adult birds were observed at least once daily for signs of toxicity, injuries, illness, or mortality. At terminal adult sacrifice, the birds were sacrificed by carbon dioxide gas asphyxiation, weighed and necropsied.

The following parameters were statistically analysed.

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

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

Eggs were collected twice daily (once on weekends and holidays) and labelled according to parental cage and date. To prevent pathogen contamination, eggs were washed in a Kuhl Egg Washer with warm water (~110ºF) and a chlorine-based detergent (Kuhl Super CD), after which they were dipped into a solution of germicidal soap (CEVA 1-Stroke Environ) and warm water. The eggs were allowed to air dry prior to being stored in an Omnicube Egg Cooler. Egg cooler temperature averaged approximately 57ºF (14ºC) and relative humidity averaged approximately 62%. Any eggs that were not able to be set (too small, too soft, obviously broken) were recorded and discarded at collection or prior to egg set measurements. Eggs that were not cracked, used for eggshell strength and thickness measurements (Section 3.7.3) were placed into a NatureForm Incubator.

Eggs were removed from the egg cooler weekly and candled to detect shell cracks. Cracked eggs were recorded and discarded. Eggs were placed into a NatureForm Incubator. The transfer of the eggs to the incubator was referred to as "egg set". Incubator temperature averaged approximately 99ºF (37ºC) with an average relative humidity of approximately 62%. The incubator was equipped with fans that circulated the air to minimise intra cabinet temperature and humidity variation during incubation. In order to prevent adhesion of the embryo to the shell membrane, the incubator was equipped with an automatic egg rotation device designed to rotate the eggs approximately every two hours. Eggs were candled again on day 14 of incubation to determine embryo viability (fertility) and on day 21 to determine embryo survival. Nonfertile and nonviable eggs were removed, recorded, and discarded.

On day 23 of incubation, the eggs were placed into a NatureForm Hatcher and allowed to hatch. The temperature and humidity are monitored at one point in the hatcher. Minor differences in temperature and humidity from the set point are to be expected

in a properly functioning hatcher. Hatcher temperature averaged approximately 99ºF (37ºC) with an average relative humidity of approximately 65%. The hatching trays were compartmentalized in order to keep hatchlings separated by parental cage. Hatchlings were removed from the hatching compartments on days 27 and 28 of incubation. They were wing banded and weighed. Wing bands made it possible to identify hatchlings to a specific parental cage. The hatchlings were group housed in brooders according to week of hatch and parental treatment group. The unhatched eggs per parental cage were observed for embryo attempts to hatch (pipping), recorded and discarded on day 28.

All eggs laid on one day every other week during the egg laying period were collected for eggshell strength and thickness measurements (lot B, D, F, H and J). Eggshell strength measurements were taken at one point on the waist using a Chatillon Model DFG Digital Force Gauge calibrated to 0.01 kg units. Following eggshell strength measurements, eggs were opened at the waist, contents removed, and the eggshells thoroughly washed. Eggshells were allowed to air dry for a minimum of 48 hours at room temperature. The average thickness of the dried shell plus the membrane was determined by measuring three points around the waist of the egg using a Mitutoyo Spherical Anvil micrometer. Measurements were made to the nearest 0.001 mm. Hatchling body weights were measured and recorded on the day they were removed from the hatcher and on day 14. Feed consumption for the offspring was not monitored. Hatchlings were observed once daily throughout the 14-day period for signs of toxicity, injuries, illness, and mortality. A record was maintained of all observations. Mortalities that occurred prior to the end of the 14-day period were recorded and discarded. Hatchlings that survived the 14-day observation period were sacrificed by CO_2 asphyxiation, weighed, and discarded.

The following parameters were statistically analysed.

Eggs Laid (mean): Analysed as the total egg production. Eggs laid will be analysed on a per hen basis.

Eggs Cracked (mean): Cracked eggs are determined by candling prior to incubation. Cracked eggs will be analysed on a per hen basis.

Eggs Set (mean): Eggs placed under incubation are "set." Analysed as the total number during the dosing phase. Eggs set will be analysed on a per hen basis.

Viable Embryos (mean): Viability (fertility) is determined by candling on the 11th day of incubation. Viability (fertility) analysed as total viable embryos. Viable embryos were analysed on a per hen basis.

Live Embryos (mean): Live embryos are determined by candling on the 18th day of incubation. Analysed as the total number of live embryos on a per hen basis.

Eggs Not Cracked of Laid (%): Eggs laid that were not cracked as a percentage of eggs laid on a per hen basis. Analysed as the mean percentage during the reproductive phase.

Eggs Set of Laid (%): Eggs set as a percentage of eggs laid on a per hen basis. Analysed as the mean percentage during the reproductive phase.

Viable Embryos of Eggs Set (%): Viable embryos as a percentage of eggs set on a per hen basis. Analysed as the mean percentage during the reproductive phase.
Live Embryos of Viable Embryos (%): Live embryos as a percentage of viable embryos on a per hen basis. Analysed as the mean percentage during the reproductive phase.
Eggshell Strength: The force needed to penetrate the shell and membrane measured at one point on the waist of the egg, analysed on a per hen basis.
Eggshell Thickness: The thickness of the shell plus the membrane measured at three points around the waist of the egg, analysed on a per hen basis.
Number Hatched (mean): Live hatchlings that had liberated themselves from their eggs by day 28 of incubation. Analysed as the total number of normal hatchlings on a per hen basis.
14-day-old Survivors (mean): Live chicks at 14-days post hatch. Analysed as the total number of 14-day survivors on a per hen basis.
Number Hatched of Eggs Laid (%): Hatchlings as a percentage of eggs laid on a per hen basis. Analysed as the mean during the reproductive phase.
Number Hatched of Eggs Set (%): Hatchlings as a percentage of eggs set on a per hen basis. Analysed as the mean during the reproductive phase.
Number Hatched of Live Embryos (%): Hatchlings as a percentage of live embryos on a per hen basis. Analysed as the mean during the reproductive phase.
14-day-old Survivors of Eggs Set (%): Live chicks at 14-days post hatch as a percentage of eggs set on a per hen basis. Analysed as the mean during the reproductive phase.
14-day-old Survivors of Number Hatched (%): Live chicks at 14-days post hatch as a percentage of hatchlings on a per hen basis. Analysed as the mean during the reproductive phase.
Hatchling Body Weight: Individual weights of the live hatchlings taken upon removal from the hatcher, analysed on a per hen basis. Analysed as the overall mean during the reproductive phase.
14-day-old Survivor Body Weight: Individual weights of the 14-day-old offspring taken at sacrifice, analysed on a per hen basis. Analysed as the overall mean during the reproductive phase.

Results and Discussion

A. Findings

Test conditions met all validity criteria, given by the mentioned guidelines.
The mean measured concentrations for the 80, 240 and 720 mg/kg feed nominal dose levels were 89, 269 and 869 mg a.s./kg feed, respectively. The control feed was also analysed and was less than the limit of quantification (< 8.75 mg a.s./kg feed) in all cases.
The daily dietary doses, based upon these measured concentrations, were 0, 9, 22 and 50 mg a.s./kg bw/day. All subsequent observations will refer to the mean measured concentrations in mg a.s./kg feed (ppm).

A total of four adult mallard died during the exposure period. One in the control group, one in the 269 mg a.s./kg treatment level and two in the 869 mg a.s./kg treatment level (prior to week 12 adult sacrifice). There was no significant difference in adult mortality as compared to the control at any treatment level. The NOEC was 269 mg a.s./kg and the LOEC was greater than 269 mg a.s./kg.

Postmortem Examination Results for Mallard Fed BYI 08330 in the Diet

Sex	Observation at Necropsy	Measured Dietary Concentration (mg a.s./kg feed)			
		Control	89	269	869
		(Number with Observation/Number Examined)			
Male	Found Dead	0/15	0/15	1/15	1/15
	Sacrificed	1/15	0/15	0/15	14/15*
	Feather Loss	0/15	0/15	0/15	1/15
	Gross Lesions	7/15	11/15	9/15	12/15
	Regressed Testes	3/15	4/15	10/15	3/15
	Skin Lesions/Abrasions	7/15	11/15	14/15	13/15
	Post Mortem Autolytic Signs	0/15	2/15	1/15	4/15
	Emaciated	0/15	0/15	2/15	7/15
Female	Found Dead	1/15	0/15	0/15	2/15
	Sacrificed	0/15	0/15	1/15	13/15*
	Feather Loss	1/15	0/15	1/15	0/15
	Gross Lesions	12/15	12/15	8/15	10/15
	Skin Lesions/Abrasions	12/15	14/15	9/15	10/15
	Regressed Ovaries	7/15	7/15	12/15	10/15
	Post Mortem Autolytic Signs	2/15	5/15	5/15	6/15
	Emaciated	0/15	0/15	1/15	6/15

*All remaining adult birds at the 869 mg a.s./kg treatment level were sacrificed 12 weeks post study initiation.

Statistical Results by Test Parameter

Parameter	**Control**	**89 ppm**	**269 ppm**	**869 ppm**	**NOEC/ LOEC**
Eggs laid/pen	50.5	51.1	28.5*	ND[a]	89 ppm 269 ppm
Eggs cracked/pen	0.14	0.07	0.13	ND	269 ppm >269 ppm
Eggs not cracked/eggs laid (%)	99.7	99.8	99.6	ND	269 ppm >269 ppm
Eggs set/pen	48.1	50.5	25.4*	ND	89 ppm 269 ppm

Shell thickness	0.364	0.364	0.344	ND	269 ppm >269 ppm
Eggshell strength	2.64	2.80	2.53	ND	269 ppm >269 ppm
Eggs set/eggs laid (%)	88.4	92.2	87.1	ND	269 ppm >269 ppm
Viable embryo/pen	40.0	25.2	16.1*	ND	89 ppm 269 ppm
Viable embryos/eggs set (%)	79.4	62.3	54.1	ND	269 ppm >269 ppm
Live embryos/pen	39.6	40.8	23.3*	ND	89 ppm 269 ppm
Live embryo/viable embryo (%)	98.9	99.2	94.9*	ND	89 ppm 269 ppm
No. of hatchlings/pen	27.8	32.2	8.1*	ND	89 ppm 269 ppm
No. of hatchlings/eggs laid (%)	48.5	56.6	20.7*	ND	89 ppm 269 ppm
No. of hatchlings/eggs set (%)	54.5	61.1	23.0*	ND	89 ppm 269 ppm
No. of hatchlings/live embryo (%)	69.5	80.4	32.2*	ND	89 ppm 269 ppm
Hatchling survival/pen	29.7	32.2	8.9*	ND	89 ppm 269 ppm
Hatchling survival/eggs set (%)	54.5	61.1	23.0*	ND	89 ppm 269 ppm
Hatchling survival/no. of hatchlings (%)	99.2	100.0	99.5	ND	269 ppm >269 ppm
Hatchling weight (g)	37.2	34.7[b]	29.0*	ND	89 ppm 269 ppm
Survivor weight (g)	260.6	250.8	217.4*	ND	89 ppm 269 ppm
Mean food consumption (g/bird/day)	113	118	86*	57*[c]	89 ppm 269 ppm
Male weight gain (g)	115	5	-113*	-315*[c]	89 ppm 269 ppm
Female weight gain (g)	99	140	-8*	-124*[c]	89 ppm 269 ppm

*A significant statistical difference was determined for these reproductive data per hen at the 269 mg a.s./kg treatment level as compared to the controls.

[a] ND = Statistical analysis of the reproductive data were not determined for the 869 mg a.s./kg treatment level due to adult sacrifice 12 weeks post study initiation.

[b] A statistically significant difference was determined at the 89 mg a.s./kg treatment level for hatchling body weight.

However due to an approximate 6% reduction of body weight from the control hatchlings and that the 89 mg a.s./kg hatchling body weights recovered by day 14, the statistical difference was not considered biologically relevant.
[c] Adult mallard data for this group up to and including week 12 post study initiation.

B. Observations

Approximately week 7 post study initiation, nine of the thirty adult mallard in the 869 mg a.s./kg treatment level began to express signs of ataxia (loss of balance). The birds in this treatment level were also noted to have severe peeling and cracking of the foot webbing. During study week 7, one hen was found dead. By study week 8, nineteen of the remaining twenty eight birds in the 869 mg a.s./kg treatment level had severe peeling and cracking on the bottoms of their feet. The birds could not feed or drink and by week 9, body weights of these adult birds showed a marked decrease from week 1 body weights. The foot symptoms were originally diagnosed as "bumblefoot" by the attending veterinarian although they were noted to be atypical of this foot condition which is occasionally noted in laboratory-housed mallards. A foot treatment of 3% hydrogen peroxide was provided to the 869 mg a.s./kg treatment level birds to reduce the foot symptoms prior to reproductive phase. At week 12, two more adult mortalities occurred and approximately 79% of the remaining 24 birds were observed with moderate to severe foot symptoms. All of the adult mallard in the 869 mg a.s./kg treatment level were sacrificed at the end of week 12 post study initiation for humane reasons due to the condition of the feet resulting in emaciation.

The severe peeling and cracking of the mallard feet were observed in one bird at the 269 mg a.s./kg treatment level beginning week 9 post study initiation. By adult sacrifice (week 21), approximately 54% of the 269 mg a.s./kg study birds had the foot condition. One bird mortality occurred during this time period. Adults were able to feed and drink, did not show the same signs of ataxia as seen in the 869 mg a.s./kg treatment level and were able to complete the reproductive phase of the study. Adult body weights at termination for both the male and female birds were statistically different from the control group at this treatment level.

A light to moderately severe form of the peeling and cracking foot symptoms were observed in one bird at week 14 post study initiation at the 89 mg a.s./kg treatment level. At the time of adult sacrifice (week 21) in the 89 mg a.s./kg treatment level, approximately 27% of the adult birds showed foot symptoms. No bird mortalities occurred at this treatment level and the adult body weights at termination were not statistically different from the control birds.

At week 16 post study initiation in the control group, one adult mallard was observed to have a bumblefoot-like condition. At adult sacrifice (week 21), six of twenty eight (one mortality occurred) control birds were indicated as having bumblefoot-like foot symptoms. These symptoms, however, were not the peeling and cracking foot symptoms that was seen in the 269 and 869 mg a.s./kg treatment levels. These symptoms were indistinguishable from foot cage wear that is typically seen due to housing conditions.

Excluding the foot symptoms that were indistinguishable from cage wear or "typical' bumblefoot, the foot symptoms appeared related to dose. However, it was unclear whether the foot symptoms had an effect on the outcome of the other endpoints analysed for this study. The NOEC based on foot symptoms was < 89 mg a.s./kg and the LOEC was 89 mg a.s./kg. However, because behavioural and body weight effects were not noted at this level, the effect was not considered adverse. The NOAEC was 89 mg a.s./kg feed.

At the conclusion of the study, another mallard reproduction study, EBFNX083, was initiated to further evaluate the foot symptoms and derive a NOEC for both the foot effects and the conventional avian reproduction endpoints. The reoccurrence of the foot symptoms in the subsequent study led to substantial guideline deviations in order to preserve bird health through test termination (including topical antiseptic treatment, non-random redistribution of cages in the study room, and increased bird handling). These modifications, made prior to and during this study, compromised the interpretability of the data. Therefore, the second study was deemed inconclusive and of questionable reliability. This study is briefly summarized and raw data included in the report.

The offspring were also observed for clinical symptoms during the 14-day growth period. There were no hatchlings produced from the entire study that were observed to have any abnormal symptoms. A total of 4 hatchlings were found dead during the observation period. None of these deaths were compound related. The NOEC was 269 mg a.s./kg and the LOEC was greater than 269 mg a.s./kg.

CONCLUSION

Although there were foot effects and a statistically significant reduction in hatchling body weight at the 89 mg a.s./kg feed level, these effects were not considered adverse or biologically significant. After a review of all parameters, the NOAEC for adult mallard exposed to technical BYI 08330 in the diet was 89 mg a.s./kg feed or 9 mg a.s./kg bw/day. The LOAEC was 269 mg a.s./kg feed or 22 mg a.s./kg bw/day based on effects on multiple parental and reproductive endpoints including adult body weight gain and food consumption, egg production, hatching success, hatchling weight and number of 14-day survivors.

Report:	**KIIA 8.1.4/03**, [redacted].; [redacted].; [redacted], [redacted] **2006**
Title:	BYI 08330 Technical: A Reproduction Study with the Mallard. Date: 2006-09-25
Organisation:	[redacted], USA
Report No.:	EBFNP024
Publication:	Unpublished
Dates of experimental work:	February 24, 2006-September 7, 2006
Guidelines:	USEPA FIFRA 71-4 OPPTS 850.2300 (Draft)

	OECD 206 Avian Reproduction Test ASTM "Standard Practice for Conducting Reproductive Studies with Avian Species"
Deviations:	None that affected study quality
GLP:	yes

Executive summary

This study is a one-generation reproduction study design with the purpose to evaluate the effects of dietary exposure to technical BYI 08330 on the health and reproductive capacity of adult mallard ducks (*Anas platyrhynchos*) as a surrogate species. Effects on adult health, weight gain, feed consumption, and reproductive parameters were monitored and evaluated and expressed as NOEC and LOEC.

Adult mallards were exposed to nominal dose levels of BYI 08330 technical grade active ingredient at dose levels of 0 (control), 30, 80, 200, and 720 mg a.s./kg feed (nominal) for 12 weeks prior to egg-laying and for 10 weeks during the reproductive phase of the study. Body weight, feed consumption, clinical appearance, reproductive parameters, mortality, and post mortem findings of the parental generation were monitored. Reproductive parameters included egg production, egg fertility, embryo survival, hatchability, hatchling body weight, 14-day survival, 14-day survivor body weight, and eggshell strength/thickness.

The reported results refer to the nominal concentrations of BYI 08330 in feed (30, 80, 200, 720 mg a.s./kg feed).

Adult parameters: A statistically significant effect was determined at the 200 mg a.s./kg treatment level for female body weight at study termination and female body weight change for both periods 1) between week 9 and adult termination and 2) total body weight change for the study period. Treatment-related foot lesions characterised by thickened areas that appear rough, dry and hard, with cracking, fissuring, soreness and/or bleeding evident (TRSF) were also present. The NOEC was 80 mg a.s./kg feed.

Egg parameters: A statistically significant effect was determined at the 200 mg a.s./kg treatment level for eggs laid/max laid, viable embryos/eggs set, percent live embryos of viable. The NOEC was 80 mg a.s./kg feed.

Hatchling parameters: A statistically significant effect was determined at the 200 mg a.s./kg treatment level for the hatchling survival/max set, hatchling survival/eggs set, number hatched/live embryos, number hatched/eggs set, number hatched/max set, hatchling weight, and survivor weight. A statistically significant difference was determined at the 80 mg a.s./kg treatment level for hatchling body weight (<10% decrease). Therefore, the NOEC was 30 mg a.s./kg feed.

Clinical Observations and Gross Pathology: Treatment-related findings included lesions on the bottoms of the feet characterised by thickened areas that appeared rough, dry and hard, with cracking, fissuring and/or bleeding evident. Despite close examination of all external surfaces,

lesions of this nature were observed only on the bottoms of the feet. Foot symptoms at 720 mg a.s./kg treatment levels were in some cases severe enough to impair normal standing and feeding behaviour. Additionally, the numbers of hens with regressed ovaries and the numbers of drakes with reduced testes (≤ 4.0 cm) were increased in both the 80 and 200 ppm a.s. treatment groups.

Conclusions: After a review of all parameters, the NOEC for adult mallard exposed to technical BYI 08330 in the diet was 30 mg a.s./kg feed or 4.2 mg a.s./kg bw/day. The LOEC was 80 mg a.s./kg feed or 10 mg a.s./kg bw/day based on effects on multiple parental and hatchling endpoints including female body weight gain, foot symptoms, and hatchling weight.

MATERIAL AND METHODS

A Materials

1. Test material — **Spirotetramat (BYI 08330) tech.**

Description — **White powder**

Lot/batch No. — **Batch no.: 08045/0014**

Purity — **97.99%**

Stability of test compound — **Expiration date: April 18, 2007**

2. Vehicle and/or positive control — **Acetone**

3. Test animals

Species — **Adult mallard ducks (*Anas platyrhynchos*)**

Age — **19 weeks of age; approaching first breeding season**

Source — **[redacted], USA**

Acclimation period — **4 weeks**

Replicates — **15 breeding pairs per dose level; one male and one female per pen**

Environmental conditions

Temperature — **Adults –**

	Mean Temperature (^{0}C)	% Relative Humidity
Control	23.3 ± 0.6	49.9 ± 21.8
30 ppm a.s.	21.9 ± 1.3	61.1 ± 18.8
80 ppm a.s.	21.8 ± 1.2	64.5 ± 19.8
200 ppm a.s.	22.8 ± 0.7	54.0 ± 21.1
720 ppm a.s.	21.5 ± 0.5	27.3 ± 9.7

Hatchlings – Petersime Hatcher 37.2 ± 0.0°C (SD) with a wet bulb temperature of 33.3 ± 0.1°C (SD NatureForm Hatcher 37.4 ± 0.0°C (SD) with a relative humidity of 58.5 ± 2.1% (SD)

	Brood compartments were 38° C from the time of hatching until the birds were five to seven days of age, when the temperature was adjusted to maintain a temperature of approximately 29°C average relative humidity of 75.3 ± 6.0% (SD). The photoperiod for the hatchlings was maintained by a time clock at 16 hours of light per day.
Photoperiod	**Adults** **Acclimation and pre-reproductive phase - 8 hours light or less of light** **Reproductive phase - 17 hours light/7 hours dark** **Light intensity was 21 foot candles provided by fluorescent light which closely approximated noon-day sunlight** **Hatchlings** **16 hours light/8 hours dark**

B Study design and methods

1. In life dates **March 1, 2005-September 7, 2006**

2. Experimental treatments

Adult birds were identified by individual leg bands, each pen was identified with a unique number and groups of pens were identified by project number and concentration. All eggs laid during the study were marked with the pen number using a soft lead pencil or permanent ink marking pen for identification. Hatchlings were identified by wing bands so that they could be traced to their parental pen of origin. The mean body weight and standard deviation for all adults of each sex was determined. All birds were 19 weeks of age at test initiation (first day of exposure to test diet) and ranged in weight from 853-1355 grams at test initiation.

All adult birds and their offspring were given feed and water *ad libitum* during acclimation and testing. The basal diet fed to both adults and offspring was formulated to [redacted] specifications by [redacted]. The basal ration contained at least 27% protein and 2.5% fat, and no more than 3.8% crude fiber. An additional 5% (w/w) of limestone (approximately 38.5% Ca) was added to the basal diet for the adults to ensure proper egg shell formation. This raised the calcium level in the diet for the breeding birds to approximately 3%, slightly above the minimum recommended for quail (2.3%) and mallard (2.75%). Offspring received basal diet without test substance and without the addition of 5% supplemental limestone.

Four of the five groups were administered treated diets containing nominal concentrations of 30, 80, 200, or 720 mg a.s./kg feed during the study. The fifth group,

designated as the control group, was administered untreated feed. The offspring produced during the study were housed according to parental treatment group.
The compound was mechanically mixed into the adult feed using an acetone carrier. Feed mixing was performed approximately once per week.
The measured concentration of BYI 08330 was determined in the treated diet.
Homogeneity of the test substance in the diet was evaluated by collecting six samples from each of the treated diets and one sample from the control diet on Day 0 of Week 1. Samples were collected from the top, middle and bottom of the left and right sections of the mixing vessel. Control and treatment group diet samples were also collected from the bin feeders on Day 7 of Week 1 to assess stability of the test substance under actual test conditions. Additionally, one sample from the control group diet and two samples from each treatment group diets were collected during Weeks 2, 3, 4, 8, 12, 16 and 20 of the test to measure/verify test concentrations.
Upon completion of the test, an analysis of variance (ANOVA) was performed to determine statistically significant differences between groups. Dunnett's multiple comparison procedure was used to compare the three treatment means with the control group mean and assess the statistical significance of the observed differences. Sample units were the individual pens within each experimental group, except adult body weights where the sample unit was the individual bird. Percentage data were examined using Dunnett's method following arcsine square root transformation.

Each of the following parameters was analysed statistically:
1. Adult Body Weight - Individual body weight was measured at test initiation, Weeks 2, 4, 6, 8 and at adult termination. Statistical comparisons were made between the control group and each treatment group at each weighing interval by sex.
2. Adult Feed Consumption - Feed consumption expressed as grams of feed per bird per day was examined weekly by weekly during the test. Statistical comparisons were made between the control and each treatment group.
3. Eggs Laid of Maximum Laid - The number of eggs laid per female divided by the largest number of eggs laid by any one female. This transformation was used to convert the number of eggs laid to a percentile value less than or equal to 100.
4. Eggs Cracked of Eggs Laid - The number of eggs determined by candling to be cracked divided by the number of eggs laid, per pen.
5. Viable Embryos of Eggs Set - The number of viable embryos at the Day 14 candling was divided by the number of eggs set, per pen.
6. Live 3-Week Embryos of Viable Embryos - The number of live embryos at the Day 21 candling was divided by the number of viable embryos, per pen.
7. Hatchlings of 3-Week Embryos - The number of hatchlings removed from the hatcher was divided by the number of live 3-week embryos, per pen.
8. 14-Day Old Survivors of Hatchlings - The number of 14-day old survivors was divided by the number of hatchlings, per pen.
9. Hatchlings of Eggs Set - The number of hatchlings was divided by the number of eggs set, per pen.

10. 14-Day Old Survivors of Eggs Set - The number of 14-day old survivors was divided by the number of eggs set per pen.
11. Hatchlings of Maximum Set - The number of hatchlings per female divided by the largest number of eggs set from any one female. This transformation was used to convert the number of hatchlings to a percentile value equal to or less than 100.
12. 14-Day Old Survivors of Maximum Set - The number of 14-day old survivors per pen divided by the largest number of eggs set.
13. Egg Shell Thickness - The average egg shell thickness of indiscriminately selected eggs per pen was measured.
14. Offspring's Body Weight - The group body weights of surviving hatchlings and 14-day old survivors were measured by parental pen group.
3. Observations
All adults and offspring were observed at least once daily for mortality, general condition, overt signs of toxicity and abnormal behaviour.

Animal Body Weights/Feed Consumption
Individual body weights of the adults were taken at the initiation of the test, at the end of test weeks 2, 4, 6, 8, 9 and at adult termination.
Feed consumption for each pen was measured weekly throughout the test. Feed consumption was determined by weighing the freshly filled feeder on Day 0, recording the amount of any additional diet added during the week, and weighing the feeder and remaining feed at the end of the feeding period (Day 7). The amount of feed wasted by the birds was not quantified, since the wasted feed was normally scattered and mixed with water and excreta. Therefore, feed consumption is presented as an estimate of total feed consumption.

Necropsy
Adult birds that died or were euthanized during the course of the study were subjected to a gross necropsy. At the conclusion of the exposure period, all surviving adult birds were euthanized by cervical dislocation, necropsied, and disposed of by incineration. At necropsy, the feet from each bird were severed distal to the hock and placed in buffered formalin for potential histopathalogical evaluation.

Egg Collection and Storage
Eggs were collected daily from all pens, when available. Eggs to be incubated were washed to reduce the possibility of pathogen contamination before storing them in the cold room. Eggs were washed in a commercial egg washer (Kuhl Egg Washer) with a chlorine based detergent (Kuhl Super CD). Water in the washer was warmed to approximately 45°C. The eggs were placed in the wash water and soaked for approximately 15 seconds. The washer's circulation motor was then turned on for approximately three minutes. The eggs were removed from the washer, allowed to cool to approximately room temperature and rinsed with fresh water. The eggs were then stored in a cold room until incubation. The cold room was maintained at a mean

temperature of 14.0 ± 0.0°C (SD) with a mean relative humidity of approximately 45 ± 0.0% (SD). Groups of eggs were identified by an alphabetic lot code. All eggs laid in a weekly interval were considered as one lot.

Candling and Incubation

At the end of the weekly interval, all eggs were removed from the cold room, counted and eggs selected by indiscriminate draw for egg shell thickness measurement. The remaining eggs were candled with a Speed King (Model No. 32) egg-candling lamp to detect egg shell cracks or abnormal eggs. Cracked or abnormal eggs were recorded and discarded.

All eggs not discarded or used for egg shell thickness measurements were placed in a NatureForm Incubator (Model No. NMC 7000). The incubator was equipped with a pulsator fan and blades that produced a mild breathing air movement designed to eliminate intracabinet temperature and humidity variation during incubation. In order to prevent adhesion of the embryo to the shell membrane, the incubator was also equipped with an automatic egg rotation device, designed to rotate the eggs from 50° off of vertical in one direction to 50° off of vertical in the opposite direction (total arc of rotation was 100°) every two hours through Day 24 of incubation. Eggs were candled on Day 14 of incubation to determine embryo viability and on Day 21 to determine embryo survival.

Hatching and Brooding

On Day 24 of incubation, the eggs were placed in a Petersime Hatcher (Model No. SP6H) or a NatureForm Hatcher (Model No. NMC-4000) and allowed to hatch. Pedigree baskets constructed of galvanized steel wire mesh were used to keep hatchlings separated by parental pen of origin. Eggs were not rotated in the hatcher. All hatchlings, unhatched eggs and egg shells were removed from the hatcher on Day 27, 28, 29 or 30 of incubation. The group body weight of the surviving hatchlings by pen was determined. Hatchlings were wing banded for identification by pen of origin and then routinely housed according to the appropriate parental concentration grouping in brooding pens until 14 days of age. At 14 days of age, the average body weight by parental pen of all surviving ducklings was determined. The ducklings were euthanized with carbon dioxide and disposed of by incineration.

Hatchlings were housed in batteries of brooding pens manufactured by Safeguard Products, Inc. Each pen measured approximately 62 x 92 x 25.5 cm high. The walls, floors and ceilings of each pen were constructed of vinyl-coated wire mesh.

Egg Shell Thickness Measurements

Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered pens during odd numbered weeks (1,3,5, etc.) and from each of the even numbered pens during the even numbered weeks (2,4,6, etc.). The eggs were opened at the waist, the contents removed, and the shells thoroughly rinsed with water. The shells were then allowed to air dry for at least one week at room

temperature. The average thickness of the dried shell plus the membrane was determined by measuring five points around the waist of the egg using a micrometer. Measurements were made to the nearest 0.002 mm.

RESULTS AND DISCUSSION

A. Findings

None of the control samples showed any indication of the presence of the test substance or of the presence of a co-eluting substance at the characteristic retention time of the test substance. Samples collected during the test to verify test substance concentrations for the 30, 80, 200 and 720 ppm a.s. diets had means and standard deviations of 28.7 ± 1.26 ppm a.s., 81.0 ± 3.99 ppm a.i, 197 ± 14.1 ppm a.s. and 752 ± 24.4 ppm a.s., respectively. The coefficients of variation were 4.40%, 4.93%, 7.17% and 3.24%. These values represented 96%, 101%, 99% and 104% of nominal concentrations, respectively. Analytical data confirmed BYI 08330 diet homogeneity, and room stability.

The daily dietary doses, based upon nominal concentrations and food consumption, were 0, 4.2, 10 and 23.6 mg a.s./kg bw/day (corresponding to 0, 30, 80, and 200 ppm). All subsequent observations will refer to the nominal concentration in mg a.s./kg feed (ppm).

No mortalities occurred in the control group or in the 30 or 80 ppm a.s. treatment groups. A single mortality occurred in the 200 ppm a.s. treatment group, the female in Pen 348 was found dead on Day 0 of Week 21. During Week 10 the hen had been noted with cracking lesions on the bottoms of both feet, but immediately prior to death the bird was normal in appearance and behaviour. At necropsy, the bird was emaciated, with a body weight of 508 g, loss of muscle mass and prominent keel. The bottoms of the hen's feet exhibited lesions of bumblefoot, but cracking was also noted. Internally, the bird had no coronary fat, the spleen was small and pale, the kidneys were pale and the ovary regressed. No other mortalities occurred in the 200 ppm a.s. test concentration during the course of the study.

B. Observations

On Day 3 of Week 5, as a result of clinical observations and loss of condition noted in the 720 ppm a.s. treatment group, all birds in that group were euthanized with carbon dioxide and subjected to gross necropsy. Birds in the 720 ppm a.s. treatment group were first noted exhibiting clinical signs of toxicity, which ultimately included loss of coordination, lower limb weakness and cracked and bleeding lesions on the bottoms of their feet, on Day 3 of Week 3. Marked, continued loss of body weight was also apparent at the Week 4 body weight interval.

Incidental clinical observations noted during the test included those that normally are associated with injuries and penwear. Such signs included bumblefoot (plantar pododermatitis), lameness, feather loss, molting and neck, back and shoulder lesions.

All adult birds were subjected to gross necropsy following termination. Treatment related findings included lesions on the bottoms of the feet characterised by thickened areas that appeared rough, dry and hard, with cracking, fissuring and/or bleeding evident. These lesions were noted in four males and six females from the 80 ppm a.s. treatment group, 13 males and 14 females from the 200 ppm a.s. treatment group and 14 males and 12 females from the 720 ppm a.s. treatment group. Despite close examination of all external surfaces, lesions of this nature were observed only on the bottoms of the feet. Additionally, the numbers of hens with regressed ovaries and the numbers of drakes with reduced testes (≤ 4.0 cm) were increased in both the 80 and 200 ppm a.s. treatment groups. Finally, a number of birds from the 720 ppm a.s. treatment group were noted to be thin, with loss of muscle mass. All other findings were considered unrelated to treatment.

There were no apparent treatment-related effects on feed consumption at the 30 ppm a.s. test concentration. At the 80 ppm a.s. test concentration, there was a slight, statistically significant ($p < 0.05$) reduction in mean feed consumption during Week 1, that, while transient, may have been related to treatment. At the 200 ppm a.s. test concentration, there were treatment-related reductions in feed consumption during Week 4 and from Week 11 through 18 that were statistically significant at $p < 0.05$ and 0.01. At the 720 ppm a.s. test concentration there were treatment-related reductions in feed consumption from Week 1 to Week 5 (termination of the adult birds for the high dose group) that were statistically significant at $p < 0.01$.

Postmortem Examination Results for Mallard Fed BYI 08330 in the Diet

Sex	Observation at Necropsy	Measured Dietary Concentration (mg a.s./kg feed) Control	30	80	200	720
		(Number with Observation/Number Examined)				
Male	Found Dead	0/15	0/15	0/15	0/15	0/15
	Sacrificed	0/15	0/15	0/15	0/15	15/15 [a]
	Foot lesions-TRSF[b]	0/15	0/15	4/15	13/15	14/15
	Foot Lesions – Bumblefoot	7/15	15/15	10/15	2/15	9/15
	Testes Small	6/15	8/15	12/15	12/15	0/15
	Regressed Ovary			-	-	-
Female	Found Dead	0/15	0/15	0/15	1/15	0/15
	Sacrificed	0/15	0/15	0/15	0/15	15/15 [a]
	Foot Lesions - TRSF	0/15	0/15	6/15	14/15	12/15
	Foot Lesions – Bumblefoot	15/15	14/15	13/15	3/15	7/15
	Testes Small			-	-	-
	Regressed Ovary	4/15	3/15	9/15	11/15	0/15

[a]All remaining adult birds at the 720 mg a.s./kg treatment level were sacrificed 5 weeks post study initiation.
[b]Foot lesions are characterised by thickened areas that appear rough, dry and hard, with cracking, fissuring, soreness and/or bleeding evident.

Statistical Results by Test Parameter

Parameter	**Control**	**30 ppm**	**80 ppm**	**200 ppm**	**NOEC/ LOEC**
Eggs laid/max laid	63	57	42	27*	80 ppm 200 ppm
Eggs cracked/laid	2	1	1	2	200 ppm >200 ppm
Shell thickness	0.389	0.382	0.389	0.373	200 ppm >200 ppm
Viable embryos/eggs set (%)	87	83	91	48*	80 ppm 200 ppm
Live embryo/viable embryo (%)	98	99	98	96	200 ppm >200 ppm

No. of hatchlings/max set	42	33	27	9*	80 ppm 200 ppm
No. of hatchlings/eggs set (%)	69	63	60	25*	80 ppm 200 ppm
No. of hatchlings/live embryo (%)	79	77	63	41*	80 ppm 200 ppm
Hatchling survival/max set	42	33	26	8*	80 ppm 200 ppm
Hatchling survival/eggs set (%)	69	62	57	23*	80 ppm 200 ppm
Hatchling survival/no. of hatchlings (%)	100	99	94	91	80 ppm 200 ppm
Hatchling weight (g)	34	32	31*	28*	30 ppm 80 ppm
Survivor weight (g)	274	266	257	200*	80 ppm 200 ppm
Male weight gain (g)	-32	9	4	-34	200 ppm >200 ppm
Female weight gain (g)	58	84	-2	-73*	80 ppm 200 ppm

* Statistically significant compared to control

CONCLUSION

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption at the 30 ppm a.s. test concentration. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured at the 30 ppm a.s. test concentration. At the 80 ppm a.s. test concentration there were treatment-related foot lesions and effects upon feed consumption and hatchling bodyweight. At the 200 ppm a.s. test concentration there were treatment-related foot lesions, and effects on adult and offspring body weights, feed consumption and marked effects upon reproductive parameters. Due to the debilitated condition of the birds, the 720 ppm a.s. treatment group was terminated during the fifth week of the study. The NOEC for mallard exposed to BYI 08330 technical in the diet during the study was 30 ppm a.s.

Report:	**KIIA 8.1.4/04, [redacted] (2007)**
Title:	Semi-Field Study of the Effects of Dermal and Dietary Exposure to BYI 08330 in Mallard Ducks (*Anas platyrhynchos*). Date: 2007-06-26
Organisation:	[redacted], USA
	Owner: Bayer CropScience
Report No.:	13798.4116, M-290000-01-1
Publication:	Unpublished
Dates of experimental work:	September 11, 2006 - December 18, 2006
Guidelines:	Non-Guideline Study
Deviations:	not applicable
GLP:	yes

Executive summary

This study was designed to determine whether dermal effects to the feet observed in laboratory cages will occur in Mallards exposed to BYI 08330 in a more natural outdoor, field-pen environment. In this study Mallards received dermal exposure by walking on turf (mowed grass) treated 3 times with MOVENTO® at nominal concentrations of 158 g a.s./ha, 96.8 g a.s./ha and 140 g a.s./ha (recoveries ranged from 66.4 to 90.0% of nominal) with a spray interval of 2 weeks as well as by dietary exposure from feed mixed with technical BYI 08330 at an nominal concentration of 200 mg a.s./kg feed (mean measured concentration of 179 mg a.s./kg feed).

There were three treatment replicates containing 6 males and 6 females each, all of which experienced identical exposure scenarios (dermal and dietary exposure). There also were three control replicates that were not exposed to the test substance. Birds were exposed to treated feed and turf for 10 weeks starting after the first application. At the end of this treatment period, all ducks in one cone control and one treatment pen were euthanized, and their feet removed and preserved. The remaining Mallards were fed untreated feed to recover for further 4 weeks. Daily observations were made for all animals to note any mortality or abnormal behaviour and the feed consumption. Every two weeks the feet of each bird were examined. The body weight of each bird was determined at study initiation and every two weeks thereafter.

No mortality was observed during the study. Behavioural observations included certain individuals limping and exhibiting reduced walking activity from week 8 through week 10 of the study.

Observations of abnormal dermal conditions on the feet, consisting of cracked skin on the underside of the feet (on the digits but not webbing), were first noted at two weeks following initial exposure to treated feed and turf. These conditions continued and increased in occurrence and severity through 10 weeks of exposure to treated feed and through 3 simultaneous applications of MOVENTO® to the pen turf made at 2-week intervals. Foot effects subsided substantially by 2 weeks after removal of the treated feed (replacement by clean feed), and were practically non-existent by four weeks after removal of treated feed, which was 10 weeks after the last (third) turf application.

No significant treatment-related effects on proportional change in body weights were detected during this study. Additionally, based on graphical evaluation of the data, there were no apparent treatment-related differences in food consumption among treatment groups during this study.

Conclusions: As few minor cracks were detected in various control birds throughout the study, this suggests that a certain degree of cracking is to be expected in duck feet in a field setting, and may be categorized as normal. Generally, moderate to severe cracking were not observed in control birds, and therefore are the conditions of most interest as an effect of this study's treatment.

MATERIAL AND METHODS

A Materials

1. Test material

	a) Spirotetramat (BYI 08330) tech. (used for treatment of the diet)
	b) MOVENTO® (BYI 08330 150 OD) (used for field application)
Description	a) White powder
	b) Not reported
Lot/batch No.	a) Mix Batch 08045/0014
	b) 08030/0189(0152)
Purity	a) 98.1%
	b) 14.7%
Stability of test compound	Recertification dates:
	a) November 17, 2006
	b) February 15, 2007

2. Vehicle and/or positive control — Vehicle: Basal bird feed; Positive control: none

3. Test animals

Species	Adult mallard ducks (*Anas platyrhynchos*)
Age	19 weeks and 4 days upon receipt 26 weeks and 6 days of age upon experimental start
Source	[redacted] USA
Acclimation period	2 weeks and 4 days
Replicates	18 breeding pairs per dose level; six males and six females per pen, three pens per dosing group
Environmental conditions	14 total rainfall events ranging from 0.25 to 8.38 cm collected, humidity ranged from 16 to 98%
Temperature	-3 to 31ºC
Photoperiod	Natural photoperiod

B Study design and methods

1. In life dates September 11, 2006 - December 18, 2006

2. Experimental treatments

Individually marked adult birds were randomly assigned to pens such that each pen had 6 males and 6 females. The pens were randomly assigned to either the control or treatment group. The body weight of all birds was determined.

Mallards were kept in predator-proof aviary, which had a dimension of 91.44 x 45.72 m. The aviary was evenly divided into 6 replicate pens such that each pen measured 45.72 x 15.24 m. At the west end of each pen a 9.14 m untreated zone was maintained to simulate edge or adjacent habitat. This zone was at the opposite end of the pens where the feed was placed. At the centre of the untreated zone of each pen, a small pond was constructed. The ponds measured approximately 4.57 x 6.1 x 0.61 m.

At the east end of each pen was the feeding area. This area was treated in the course of the turf spray applications. This scenario required the ducks to cross 36.6 m of treated turf from the pond area to obtain feed. Natural temperature, humidity, and lighting conditions were not supplemented in any way. Temperature and humidity were monitored by a VWR Scientific Products digital min/max thermometer/hygrometer, and extremes recorded daily. Rain events were monitored and precipitation measured as events occurred. Additionally, daily temperature records were obtained for the study duration from a local National Climatic Center weather station.

Purina® Game Bird Flight Conditioner® and water were provided to the test organisms *ad libitum*. Feed of the treatment group was treated with BYI 08330 tech. at a nominal concentration of 200 mg a.s./kg feed. Birds of the control group received untreated feed.

A typical tractor-mounted agricultural boom sprayer and PTO pump was used to apply the test substance to the turf within the treatment replicate pens, and to apply clean water to the turf within the control replicate pens. The sides of the pens were covered with plastic sheeting to prevent spray-drift into adjacent pens. Prior to each turf application, the ducks in each pen were captured and placed in holding cages located at the far end of the untreated zone in their respective pens. The ducks were released from these holding cages immediately after turf treatment in all pens was complete. The test substance was applied to the turf in the treatment replicate pens on three occasions spaced 14 days apart and at a rate of 158 g a.s./ha (Event 1), 96.8 g a.s./ha (Event 2) and 140 g a.s./ha (Event 3), respectively. The turf in each control pen was sprayed with water. Concentrations of BYI 08330 within the feed and applied to the turf were analytically verified.

Treated feed was placed in the treatment pens immediately after the turf within each pen was sprayed. The time that treated feed was first offered was considered time 0. Treated feed was provided to the treatment pens and clean (no test substance) feed to the control pens continuously from time 0 through week 10. At the end of 10 weeks, the birds in one control pen and one treatment pen were euthanized and their feet removed and preserved. Also at the end of 10 weeks, all feed in the remaining pens was replaced with clean feed. Remaining birds were left for observation through a 4-week, post-treatment recovery period during which they were fed only clean feed.

3. Observations

Daily observations were made of all animals to note any mortality or abnormal behaviour. Every two weeks the feet of each bird were examined. Descriptive terminology used in this study to describe foot condition (minor, moderate or severe cracking) were made relative to the ducks observed in this study and are not directly comparable to results noted in other studies. Foot conditions were recorded, and the feet photographed by digital camera.

Observations of duck movement within pens were made opportunistically, especially in the morning as the birds were actively foraging and moving about their pens and during food replacement activities when the birds usually would move to and from the feed supply. Notations were made to document bird travel across the treated field, and to note any abnormal behaviour. In addition, two sessions of bird behaviour and movement within the pens were captured on digital video.

Body weight was measured for each test animal on the day of treatment initiation and every two weeks thereafter.

During the test, feed consumption was measured daily, and average consumption per bird was calculated based on these measurements. Feed consumption/bird/day was calculated for the exposure and post-exposure periods by dividing the grams of feed removed from the feed containers each day by the total number of birds in the pen.

At the end of the treatment period, all ducks in one control and one treatment pen were euthanized, and their feet removed and preserved. This was to capture specimens of feet and their respective dermal condition after 10 weeks of treatment. At the end of the 4-week recovery period (4 weeks of clean feed following treatment), all ducks in one control and two treatment pens were euthanized, and their feet removed and preserved. This was to capture specimens of feet after 4 weeks of recovery. To remove the feet, the metatarsus was severed just above the foot pad, and the foot pad and digits separated. The separate pieces were placed in a common jar of formalin solution for preservation.

Statistical analysis

Measured body weights were used to calculate proportional changes in body weight by gender. These calculated values were statistically compared among pens (TOXSTAT, 1996). Data were first checked for normality using Shapiro-Wilk's Test ([redacted] 1989) and for homogeneity of variance using Levene's Test (organisation, 1994). Proportional data were anscombe arcsine transformed when appropriate (all values in dataset fall between 0 and 1) prior to analysis. Since the study design offered three control and three treatment pens, data were analyzed by analysis of variance (ANOVA) followed by a multiple comparison test to assess differences among all pens. If the data sets passed the test for homogeneity of variance and normality, ANOVA followed by Tukey's Multiple Comparison Test (TOXSTAT, 1996) was used. Data that were not normally distributed were tested via Kruskal-Wallis' ANOVA followed by Dunn's Multiple Comparison Test for mean differences among pens.

RESULTS AND DISCUSSION

A. Findings

The concentrations of BYI 08330 birds were exposed to by treated feed and turf were analytically verified. Average measured concentrations within treated feed ranged from 165.5 to 197.8 mg a.s./kg feed (82.7 to 98.9% of nominal) and resulted in a mean measured concentration of 179 mg a.s./kg feed. Since recoveries were within 80% of nominal, results of the study were based on nominal concentrations.
The results of the analyses to evaluate the concentration of the test material in the spray solutions revealed recovery rates of 66.4% to 80.7% of nominal concentration (Event 1), 78.3% to 90% (Event 2) and 67.7% to 78.7% (Event 3).

Results of the foot inspections, body weight determinations and monitoring of the food consumption are presented in the tables below.

Table 1 Summary of foot inspections, by pen, made during the semi-field study of the effects of BYI 08330 to Mallard ducks (*Anas platyrhynchos*)

		Control			Treatment		
		Pen 1	Pen 2	Pen 6	Pen 3	Pen 4	Pen 5
Treated feed	**Week 0**						
	No cracks noted	12	12	12	12	12	12
	Minor Cracks						
	Moderate Cracks						
	Severe Cracks						
	Other (i.e. callouses)	1		1	1		1
	Week 2						
	No cracks noted	5	12	11	10	8	11
	Minor Cracks	4		1	3	4	8
	Moderate Cracks						
	Severe Cracks						
	Other (i.e. callouses)				1		1
	Week 4						
	No cracks noted	9	11	6	6	6	5
	Minor Cracks	4	3	5	4	3	5
	Moderate Cracks			1	2	3	2
	Severe Cracks				1		
	Other (i.e. callouses)						1
	Week 6						
	No cracks noted	8	9	12	6	4	5
	Minor Cracks	4	3		2	3	4
	Moderate Cracks				3	4	3
	Severe Cracks				1	2	
	Other (i.e. callouses)						1
	Week 8						
	No cracks noted	10	12	10	4	4	5
	Minor Cracks	1		2	4		3
	Moderate Cracks	1			2	3	1
	Severe Cracks				2	5	3

	Other (i.e.: callouses)							
	Week 10							
	No cracks noted	12	11	11	6	8	5	
	Minor Cracks		1	1	3	2	4	
	Moderate Cracks				1	2	2	
	Severe Cracks				2	4	2	
	Other (i.e.: callouses)							
Recovery period	**Week 12**							
	No cracks noted	11		10	7		5	
	Minor Cracks	1		2	5		4	
	Moderate Cracks						3	
	Severe Cracks							
	Other (i.e.: callouses)							
	Week 14							
	No cracks noted	11		10	10		7	
	Minor Cracks	1		2	2		5	
	Moderate Cracks							
	Severe Cracks							
	Other (i.e.: callouses)							

Note: If observation was made as (i.e.) "moderate to severe", notation was included in severe tally.

Light highlighted cells indicate observations of cracks in the process of healing

Dark highlighted areas block cells for animals previously euthanized.

Table 2 Body weight summary, by pen, during the semi-field study of the effects of BYI 08330 to Mallard ducks (*Anas platyrhynchos*)

		Control pens			Treatment pens		
Gender	**Averages by pen**	**1**	**2**	**6**	**3**	**4**	**5**
Female	Time 0 mean body weight [g]	962.1	1060.9	1058.8	902.3	984.1	888.6
	Week 2 body weight [g]	1025.9	1069.1	1093.2	953.0	1056.7	925.7
	Week 0-2 proportional body weight change	0.1	0.0	0.0	0.1	0.1	0.0
	Week 4 body weight [g]	1079.8	1114.6	1089.1	1041.5	1094.7	1045.5
	Week 2-4 proportional body weight change	0.1	0.0	0.0	0.1	0.0	0.1
	Week 6 body weight [g]	1080.7	1150.3	1121.6	1024.9	1106.2	1027.9
	Week 4-6 proportional body weight change	0.0	0.0	0.0	0.0	0.0	0.0
	Week 8 body weight [g]	1145.3	1211.3	1119.3	1040.4	1100.1	1074.9
	Week 6-8 proportional body weight change	0.1	0.1	0.0	0.0	0.0	0.0
	Week 10 body weight [g]	1114.5	1187.5	1139.6	1061.6	1122.6	1094.1
	Week 8-10 proportional body weight change	0.0	0.0	0.0	0.0	0.0	0.0
	Week 12 body weight [g]	1150.8	*	1130.4	1047.0	*	1084.9
	Week 10-12 proportional body weight change	0.0	*	0.0	0.0	*	0.0
	Week 14 body weight [g]	1122.2	*	1121.0	1045.5	*	1076.6
	Week 12-14 proportional body weight change	0.0	*	0.0	0.0	*	0.0

Male	Time 0 body weight [g]	1106.4	1204.9	1132.5	1086.8	1096.9	1025.0
	Week 2 body weight [g]	1126.7	1193.0	1200.5	1122.7	1124.7	1007.4
	Week 0-2 proportional body weight change	0.0	0.0	0.1	0.0	0.0	0.0
	Week 4 body weight [g]	1178.1	1214.6	1195.1	1140.5	1172.8	1129.0
	Week 2-4 proportional body weight change	0.0	0.0	0.0	0.0	0.0	0.1
	Week 6 body weight [g]	1197.5	1239.1	1237.7	1131.5	1190.6	1139.5
	Week 4-6 proportional body weight change	0.0	0.0	0.1	0.0	0.0	0.0
	Week 8 body weight [g]	1219.0	1276.9	1267.2	1138.8	1132.4	1151.8
	Week 6-8 proportional body weight change	0.0	0.0	-0.1	0.0	0.0	0.0
	Week 10 body weight [g]	1203.5	1247.9	1190.8	1147.5	1158.2	1168.2
	Week 8-10 proportional body weight change	0.0	0.0	0.0	0.0	0.0	0.0
	Week 12 body weight [g]	1216.1	*	1177.8	1138.2	*	1170.2
	Week 10-12 proportional body weight	0.0	*	0.0	0.0	*	0.0
	Week 14 body weight [g]	1196.6	*	1176.0	1127.2	*	1164.3
	Week 12-14 proportional body weight change	0.0	*	0.0	0.0	*	0.0

* = Birds euthanized for foot specimen collection

Table 3 Average weekly food consumption [g], by pen, during the semi-field study of the effects of BYI 08330 to Mallard ducks (*Anas platyrhynchos*)

		Control pens				Treatment pens			
Phase	Week	1	2	6	Control total average [g]	3	4	5	Treatment total average [g]
Treatment	1	1088.3	1121.2	1212.3	1140.6	1052.4	1149.5	1056.0	1086.0
	2	1225.4	1142.1	1235.6	1201.0	1054.2	1099.7	557.4	903.8
	3	1186.8	1087.7	1161.0	1145.2	1100.5	1145.7	1232.8	1159.7
	4	1257.0	1240.1	1227.7	1241.6	1216.7	1168.3	1256.8	1213.9
	5	1327.4	1331.1	1191.5	1283.3	1110.1	1155.0	1203.7	1156.3
	6	1268.7	1321.4	1199.3	1263.1	1177.4	1184.6	1157.0	1173.0
	7	1296.4	1230.6	1200.1	1242.4	1175.1	1188.3	1020.0	1127.8
	8	1256.5	1213.1	1130.7	1193.4	1065.2	1081.5	1235.1	1127.2
	9	1189.4	974.2	1112.2	1092.0	1070.1	1142.6	1107.3	1106.7
	10	1205.0	1096.6	1209.4	1170.3	1147.2	1172.2	1144.4	1154.6
Treatment overall average		1228.1	1175.8	1188.0	1197.3	1116.9	1148.7	1097.0	1120.9
Recovery period	11	1224.6	*	1240.1	1232.4	1168.8	*	1184.8	1176.8
	12	1309.0	*	1183.2	1246.1	1220.0	*	1167.3	1193.7
	13	1244.3	*	1215.0	1229.6	1193.4	*	1187.3	1190.4
	14	1162.3	*	1156.7	1159.5	1164.8	*	1178.5	1171.7
Recovery overall average		1235.0	NA	1198.8	1216.9	1186.8	NA	1179.5	1183.1

* = Birds euthanized for foot specimen collection

B. Observations

Mortality and behavioural observations

In the mornings the birds were typically at the ponds bathing and preening, or foraging within the field (treatment zone). The birds quickly became habituated to the replacement of their food, and frequently came to the feeding area as their food was being replaced. Typically, the birds approached the feeding area by walking, and also walked back to the pond area after feeding. It appeared that the birds usually only flew between these areas if they were startled or in a few cases had an aversion to walking due to sore feet.

No mortalities occurred during the study. Minor abnormal behaviour was first observed in one treated male and one female duck in week 6, but were observed in most affected treatment birds (not all treatment birds developed cracked skin on their feet) in week 8. Abnormal behaviours specifically included limping, reluctance to walk, and the bird sitting down almost immediately upon landing, or stumbling while walking. Marked limping was limited to a few birds in the treatment pens, but tendency to fly rather than walk (especially from the untreated zone to the feeding area) generally increased among treatment animals as treatment progressed. Since the treated birds had taken to flying more often than controls, and because they were not forced into walking for observation purposes, the exact time when limping was subsided was not documented. However, limping had definitely ceased entirely one week after the treatment period.

Foot conditions

Abnormal foot conditions were noted by week 2 of treatment (see Table 1). The abnormalities observed were cracking of the skin along the underside of the digits of the feet. Though mild cracks are apparently normal results of wear and tear of the ducks' feet in natural environments, as supported by the occurrence of such in the control birds, the treated birds experienced more minor cracking than the controls, and also experienced moderate and severe cracking.

Among birds observed at each bi-weekly interval, the incidence of abnormal foot conditions among control birds occurred in 7.6% (week 10, n = 36) to 36.1% (week 4, n = 36) of birds in that group. The incidence of abnormal foot conditions among treated birds ranged from 29.2% (week 14, n = 24) to 63.9% (week 8, n = 36). In addition, the feet of many treated birds showed a progression over time toward moderate and severe cracking. Severe cracking included those that were deep enough to expose cartilage or bone and cause severe limping. Only two observations of moderate cracking were documented on separate occasions in the control group (one during week 4 and one during week 8), with no severe observations noted. By week 4 seven moderate and one severe cracking observations were noted in the treatment group. The number of severe cases increased until week 10. However, during the recovery period, significant improvements were made in the condition of the feet of all affected treatment animals. The severe cracking markedly decreased by week 12 to only 3 moderate cases (and 9 remaining very minor cases), and were healed by week 14, when only 7 minor cases were recorded. Signs of healing during the study, and specifically as noted during the final observations on week 14 were characterized by drying up of open wounds and new tissue forming to fill the cracks. Minor cracks, such as those observed in control ducks, often healed very rapidly and were sometimes not noticeable on the same duck just 2 weeks after their initial

notation. Therefore, observations of the healing process of minor cracks were not often recorded because they were rarely observed. During the entire study, no infection was noted in cracked feet.

Body weight

Body weight data (see Table 2) was used to calculate proportional change in body weight between each two-week measurement interval. Changes in body weight were compared among pens by gender.

Females: A significant mean difference in proportional weight change was detected during week 2 to week 4 between control pen 6 and treatment pen 5, where control pen 6 lost weight while treatment pen 5 gained weight (F = 3.2595, critical f = 2.5336, p = 0.0181). The higher increase in weight gain in pen 5 is likely due to weights recovering after a transient negative effect on food consumption from a malfunctioning feeder towards the end of week 2. In addition, since it was not a treatment pen that experienced the minor amount of weight loss (< 1%), the difference detected was not attributable to treatment. Kruskal-Wallis' ANOVA also detected a significant difference among groups during week 8 to 10, however the post hoc Dunn's Multiple Comparison test did not identify any pair-wise differences (K = 12.3809, critical k = 11.0705, p = 0.0299). Numerically, the greatest difference in proportional weight change in this data set was between control pen 1 and control pen 6 (-2.5% and +2%, respectively), again, having no relationship to treatment. This data set was also subjected to standard ANOVA with Tukey's Multiple Comparison post-hoc test, which, likewise, did not find a significant difference among groups (p = 0.28). No other significant differences in mean proportional weight change were detected during any other two-week interval.

Males: Significant differences in mean proportional weight change were detected among males at 4 measurement intervals. Between time 0 and week 2, treatment pen 5 lost significantly greater weight (-1.7%) than treatment pen 3 (+3.5%) and control pen 6 (+6.0%) (F = 5.4387, critical f = 2.5336, p = 0.0011). The transient weight loss in pen 5 was likely attributable to a malfunctioning feeder in that pen during week 2. In addition, control pen 6 gained significantly more (+6%) weight (-0.8%) than control pen 2 during the same period. Between week 2 and week 4, proportional weight gains in treatment pen 3 (+1.7%), control pen 2 (+1.8%), and control pen 6 (-0.5%) were significantly less weight than that of treatment pen 5 (+12.2%) (F = 5.7836, critical f = 2.5336, p = 0.0007). This recovery in weight by pen 5 birds during this interval was likely attributable to somewhat heavier feeding following repair of the malfunctioning feeder in pen 5 at the end of week 2. During week 6 to week 8, proportional weight gain in treatment pen 4 was significantly less (-4.8%) than that of treatment pen 5 (+2.8%) and control pen 2 (+3.0%) (K = 20.4117, critical k = 11.0705, p = 0.0010). During the same period control pen 6 gained proportionally less weight (-5.2%) than control pen 2 (+3.0%). Finally, during week 8 to week 10 Kruskal-Wallis' ANOVA detected a significant difference in proportional weight change among groups; however, Dunn's Multiple Comparison post-hoc test did not identify any specific pair-wise significant differences (K = 13.1146, critical k = 11.0705, p = 0.0223). Numerically, the greatest difference was between treatment pen 4 (+2.3%) and control pen 2 (-2.3%). Since no negative weight change was noted in the treatment pen, these results did not indicate a treatment-related effect. This data set was also

subjected to standard ANOVA with Tukey's Multiple Comparison post-hoc test, which did not detect a significant mean difference among groups (p = 0.0710).
The statistical differences noted above are inconsistent, with apparent effects being transient and not occurring in the same groups at subsequent measurement intervals. Therefore there is no pattern to suggest a significant effect upon weight change in male or female ducks attributable to the treatments applied in this study. A body weight summary is presented in Table 2.

Food consumption

Food consumption followed the same general pattern in all pens during the course of the study, though average weekly food consumption in the control pens was slightly (~100 g per pen or 10 g per bird on average) higher than in the treatment pens (see Table 3). The marked drop in average food consumption in pen 5 during week 2 was attributable to a feeder malfunction on days 4 and 5 of that week. This feeder was ultimately repaired on day 5, and feed consumption, thereafter, returned to normal. However, these two days of altered food access did have a negative, though transient effect on body weights during this interval (slight drop in average body weight) and the following interval (slight increase in average body weight).

CONCLUSION

As few minor cracks were detected in various control birds throughout the study, this suggests that a certain degree of cracking is to be expected in duck feet in a field setting, and may be categorized as normal. Generally, moderate to severe cracking were not observed in control birds, and therefore are the conditions of most interest as an effect of this study's treatment. Body weight change calculated across two-week intervals did not reveal significant differences between control and treatment pens to indicate a distinct treatment effect upon this parameter. In addition, average food consumption patterns among pens were not markedly different between the control and treatment pens, indicating food treated at 200 mg a.s./kg feed did not have a food consumption effect upon the birds either.

IIA 8.2 Fish toxicity

IIA 8.2.1 Acute toxicity of the active substance to fish

IIA 8.2.1.1 Rainbow trout (*Oncorhynchus mykiss*)

Report:	**KIIA 8.2.1.1/01, [redacted]; 2004**
Title:	Acute toxicity of BYI 08330 (tech.) to fish (*Oncorhynchus mykiss*)
	Date: 2004-12-16
Organisation:	[redacted], Germany
Report No.:	DOM 24025; M-182649-01-2
Publication:	Unpublished
Dates of experimental work:	March 15, 2004 – April 27, 2004
Guidelines:	EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985)
	OPPTS 850.1075 (Public Draft, 1996)
	Directive 92/69/EEC, C.1 (1992)
	OECD No. 203 (rev.1992)
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the acute toxicity of spirotetramat to Rainbow trout expressed as 96 h-LC_{50} for mortality. 10 fish in each test level were exposed for 96 h under static - renewal (daily) conditions to nominal (mean measured) concentrations of 0.5 (0.409), 1.0 (0.825), 2.0 (1.50), 4.0 (3.14), and 8.0 (6.44) mg test item (mean measured a.s.)/L against controls (solvent control and untreated control). Dissolved oxygen, water temperature and pH values were determined daily in each aquarium. As measurements show, the physical/chemical properties corresponded to the required values. BYI 08330 contents were analysed in all test levels every day of the exposure period, new and aged test item concentrations. Since the mean measured values were partly below 80% of nominal, the results of this study are given as mean measured concentrations

The LC_{50} (96 h) was determined to be 2.54 mg a.s./L with a 95% confidence interval from 2.11 - 3.05 mg a.s./L.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014 (mix-batch)** **Tox no.: 06344-01**
Purity	**97.4%**
Stability of test compound	**Expiration date: 2004-06-04 when stored at room temperature**
Water solubility	**33.5 mg/L (pH 4), 29.9 mg/L (pH 7), 19.1 mg/L (pH 9) (20°C)**

2. Vehicle and/or positive control	**Acetone was used as solvent for the test item.**
3. Test animals	
Species	**Rainbow trout (*Oncorhynchus mykiss*), mean body length 5.8 cm, mean body weight 2.3 g, biomass loading was 0.58 g fish/L test medium**
Age	**Juvenile**
Source	**[redacted] Germany.**
Acclimation period	**> 14 days**
Environmental conditions	
Temperature	**11.3 - 12.3°C**
Photoperiod	**16 hours light / 8 hours dark**

B Study design and methods

1. In life dates **March 15, 2004 - March 19, 2004**

2. Experimental treatments

Rainbow trout (*Oncorhynchus mykiss*), (lot F 9/03) were obtained as green eggs and milt from [redacted], Germany. The parent fish were identified to species by the supplier. They hatched in the testing facility. All test fish were held in culture tanks on a 16/8 hour light/dark photoperiod and were observed for at least 14 days prior to testing. Mortality was noted prior to the test initiation and all unsuitable fish (e.g. injured, deformed, etc.) were eliminated from the test prior to the assignment of test groups. In the 48 hour acclimation period before testing less than 5 percent of the fish died. There was no treatment of the fish necessary before and during testing.

Fish were fed daily with commercial trout food (Brutfutter Ecostart 17, [redacted], Denmark) during the acclimation period. They were not fed 48 h before and during the study.

Reconstituted water was prepared by adding salt stock solutions to demineralised water (conductivity < 0.2 µS/cm) to yield defined ionic concentrations. The water was then aerated to reach the oxygen saturation point and used for the test.

Based on a range finder, the definitive test concentrations were set at nominal 0.50, 1.00, 2.00, 4.00 and 8.0 mg test item/L. Untreated water and a solvent control were run in parallel. The test media were freshly prepared every day. Acetone was used as organic solvent, not exceeding a maximum load of 200 µL in 1 L test water.

The test aquaria were made of glass with a size of 32 x 36 x 38 cm (l x d x h). The test volumes amounted to 40 L. For every test concentration one aquarium was used. At the start of the test, ten fish were randomly introduced into each aquarium.

3. Observations

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

Dissolved oxygen, water temperature and pH values were determined daily in each aquarium, water temperature was additionally measured in the control aquarium and recorded hourly with a data logger. Analytical determinations of the active

ingredient concentrations were made in the test medium daily, before and after the renewal of the test concentrations.
When possible, the LC_{50} values and the 95%-confidence intervals were calculated every 24-hour using a computer program, which estimated the LC_{50} using one of three statistical techniques: moving average, binomial probability or Probit analysis. The appropriate method was determined according to the data characteristics.

RESULTS AND DISCUSSION

A. Findings

The test conditions met all validity criteria given by the mentioned guidelines.
Based on analytical determination of BYI 08330 (tech.) (in water by HPLC-UV, limit of Quantitation (LOQ) = 5 µg/L) mean measured values between 77% and 85% of nominal were found in all exposure levels over the whole testing period of 96 hours. Since the mean measured values were partly below 80% of nominal, the results of this study are given as mean measured concentrations, equivalent to 0.409, 0.825, 1.50, 3.14 and 6.44 mg a.s./L.
The physical/chemical properties corresponded to the required values; dissolved oxygen concentrations ranged from 89 to 99% oxygen saturation, the pH values ranged from 6.9 to 7.1 and the water temperature ranged from 11.3°C to 12.3°C in all aquaria over the whole testing period
There were neither any sublethal effects nor any mortality in the control groups. Based on mean measured concentrations of spirotetramat, the 96 h - LC_{50} was calculated by probit analysis to be 2.54 mg a.s./L. The NOEC was considered to be 0.825 mg a.s./L, the highest concentration without sublethal effects. The maximum concentration causing no mortality within the period of the test (NOLEC) was 1.5 mg a.s./L. The minimum concentration causing 100% mortality (96 h) was 6.44 mg a.s./L.

Exposure period (hours)	LC_{50} [mg a.s./L]	95% C.I. [mg a.s./L]	Method of statistical calculation
24	4.69	3.81 - 5.79	Probit analysis
48	3.48	2.75 - 4.40	Probit analysis
72	3.48	2.75 - 4.40	Probit analysis
96	2.54	2.11 - 3.05	Probit analysis

B. Observations

There were behavioural anomalies in fish caused by the test item observed over the whole exposure period in all test levels ≥ 1.5 mg a.s./L. At the test level with 1.5 mg a.s./L fish showed the following symptoms after 96 h: remained for unusually long periods on the bottom of the aquarium, showed loss of equilibrium with lateral deviation from their normal orientation, showed laboured respiration, turned in a vertical position, remained for unusually long periods at the water surface.

Cumulative mortality and behavioural observations (with a total number of 10 fish tested at each concentration)

Mean measured conc. [mg a.s./L]	4 h		24 h		48 h		72 h		96 h	
	No.	Obs.	No.	Obs.	No.	Obs.	No.	Obs.	No.	Obs.
Control	10	N	10	N	10	N	10	N	10	N
Solvent control	10	N	10	N	10	N	10	N	10	N
0.409	10	N	10	N	10	N	10	N	10	N
0.825	10	N	10	N	10	N	10	N	10	N
1.50	10	N	10	N	0 2 2 6	TF V AT N	10	N	0 1 2 1 0	TF BO,TS,AT V,AT OB,AT N
3.14	10	N	0 1 1 8	TF BO,AT AT,H N	3 2 5 0	TF BO,SR,AT, AP,DF OB,AT N	3 1 3 1 3 0	TF BO,DF BO,SR, AT,DF TS,H,AT,DF N	8 1 1 1 0	TF BO,SR,AP, DF,AT BO,AT N
6.44	10	N	6 3 1 0	TF BO,SR, AT, DF BO,SR,AT, T,KR N	10	TF	10	TF	10	TF

Abbreviations:

AP: were inactive or displayed abnormally low activity
AT: showed laboured respiration
BO: remained for unusually long periods on the bottom of the aquarium
DF: turned dark in coloration
H: were hyperactive - showed exaggerated response to stimulus or disturbance
KR: had convulsions
N: did not show any abnormal signs
OB: remained for unusually long periods at the water surface
SR: laid on their sides or backs
TF: were dead
TS: showed loss of equilibrium with lateral deviation from their normal orientation
V: turned in a vertical position
-: no observations, all fish dead

CONCLUSION

Based on mean measured concentrations of spirotetramat, the 96 h - LC_{50} was calculated by Probit analysis to be 2.54 mg a.s./L (C.I. 95%: 2.11 – 3.05 mg/L). The NOEC was considered to be 0.825 mg a.s./L, the highest concentration without sublethal effects.

IIA 8.2.1.2 Warm water fish species

Report:	**KIIA 8.2.1.2/01, [redacted]; 2004**
Title:	Acute toxicity of BYI 08330 (tech.) to fish (*Cyprinus carpio*)
	Date: 2004-11-25
Organisation:	[redacted], Germany
Report No.:	DOM 24022; M-128667-01-2
Publication:	Unpublished
Dates of experimental work:	March 01, 2004 - March 25, 2004
Guidelines:	EU 96/12 EEC C1 (1996), JMAFF 12 Nousan 8147 (2000), EPA-FIFRA § 72-1 (1982), considering also OECD 203 (1992), and OPPTS 850.1075 (1996)
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the acute toxicity of the test item to common carp (*Cyprinus carpio*), expressed as 96 h-LC_{50} for mortality. 10 fish in each test level were exposed for 96 h under static - renewal (daily) conditions to nominal (mean measured) concentrations of 1.25 (1.02), 2.50 (1.71), 5.00 (3.65), 10.0 (8.13), and 20.0 (13.4) mg test item (mean measured a.s. concentration)/L against controls. Dissolved oxygen, water temperature and pH values were determined daily in each aquarium. As measurements show, the physical/chemical properties corresponded to the required values. BYI 08330 contents were analysed in all test levels every day of the exposure period (renewal and aged test medium). Since the analytical findings were partly below 80% of nominal, the results of this study are given as mean measured concentrations.

The LC_{50} (96 h) was determined to be 2.59 mg a.s./L (C.I. was not determined due to mathematical reasons).

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014 (mix-batch)**
	Tox no.: 06344-01
Purity	**97.4%**
Stability of test compound	**Expiration date: 2004-06-04 when stored at room temperature**
Water solubility:	**33.5 (pH: 4). 29.9 (pH: 7), 19.1 (pH: 9) mg / L (20°C)**
2. Vehicle and/or positive control	**Acetone was used as solvent for the test item.**
3. Test animals	
Species	**Common carp (*Cyprinus carpio*), mean body length 4.0 cm, mean body weight 0.9 g, biomass loading was 0.23 g fish/L test medium**

Age	**Juvenile**
Source	[redacted]
	Netherlands
Acclimation period	**> 14 days**
Environmental conditions	
Temperature	**20.5°C to 23.2°C**
Photoperiod	**16 hours light / 8 hours dark**

B Study design and methods

1. In life dates **March 01, 2004 - March 05, 2004**

2. Experimental treatments

Common carp (*Cyprinus carpio*), (lot F 1/04 A) were obtained from and identified by [redacted], [redacted]; Netherlands. All test fish were held in culture tanks on a 16/8 hour light/dark photoperiod and observed for at least 14 days prior to testing. Less than 5% mortality was noted prior to the test initiation and all unsuitable fish (e.g. injured, deformed, etc.) were eliminated from the test prior to the assignment of test groups. In the 48 hour acclimation period before testing less than 5 percent of the fish died. There were no treatments of the fish necessary before and during testing.

Fish were fed daily with commercial trout food (Brutfutter Ecostart 17, [redacted], Denmark) during the acclimation period. They were not fed 48 h before and during the study.

Copper(II)sulfate was tested as positive control on common carp to demonstrate satisfying test conditions; results are provided in the report.

Reconstituted water was prepared by adding salt stock solutions to demineralised water (conductivity < 0.2 µS/cm) to yield defined ionic concentrations. The water was then aerated to reach the oxygen saturation point and used for the test.

Based on a range finder, the definitive test concentrations were set at 1.25, 2.50, 5.00, 10.0 and 20.0 mg test item/L. Untreated water and a solvent control were run in parallel. The test media were freshly prepared every day. Acetone was used as organic solvent, not exceeding a maximum load of 200 µL in 1 L test water.

The test aquaria were made of glass with a size of 32 x 36 x 38 cm (l x d x h). The test volumes amounted to 40 L. For every test concentration one aquarium was used. At the start of the test, ten fish were randomly introduced into each aquarium.

3. Observations

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

Dissolved oxygen, water temperature and pH values were determined daily in each aquarium, water temperature was additionally measured in the control aquarium and recorded hourly with a data logger. Analytical determinations of the active ingredient concentrations were made in the test medium daily, before and after the renewal of the test concentrations. In case that 100% mortality was observed in test

concentrations prior to the end of the test, the analytical determinations were made at those times.

When possible, the LC_{50} values and the 95%-confidence intervals were calculated every 24-hour using a computer program, which estimated the LC_{50} using one of three statistical techniques: moving average, binomial probability or probit analysis. The appropriate method was determined according to the data characteristics.

RESULTS AND DISCUSSION

A. Findings

Validity criteria given by the mentioned guidelines were met.

Based on analytical determination of BYI 08330 (in water by HPLC-UV, Limit of Quantitation (LOQ) = 5 µg/L) mean measured values between 69% and 83% of nominal were found in all exposure levels over the whole testing period of 96 hours. Since the analytical findings were partly below 80% of nominal, the results of this study are given as mean measured concentrations, equivalent to 1.02, 1.71, 3.65, 8.13 and 13.4 mg a.s./L.

The physical/chemical properties corresponded to the required values, dissolved oxygen concentrations ranged from 97 to 103% oxygen saturation, the pH values ranged from 6.9 to 7.2 and the water temperature ranged from 20.5°C to 23.2°C in all aquaria over the whole testing period.

There were neither sublethal effects nor any mortality in the control groups. Based on mean measured concentrations, the 96 h - LC_{50} was calculated by Probit analysis to be 2.59 mg a.s./L (C.I.95%: not determined due to mathematical reasons). The overall NOEC was found to be 1.02 mg a.s./L, the highest concentration without lethal or sublethal effects. The maximum concentration at which no mortality was observed after 96 hours (NOLEC) was 1.71 mg a.s./L. The minimum concentration causing 100% mortality after 96 hours was 3.65 mg a.s./L.

Exposure period (hours)	LC_{50} [mg a.s./L]	95% C.I. [mg a.s./L]	Method of statistical calculation
24	5.05	4.29 - 5.96	Probit analysis
48	4.59	3.71 - 5.68	Probit analysis
72	3.65	n.d.	Probit analysis
96	2.59	n.d.	Probit analysis

n.d. not determined due to mathematical reasons

B. Observations

There were behavioural anomalies in fish observed over the whole exposure period in all test levels ≥ 1.71 mg a.s./L. At the test level with 1.71 mg a.s./L fish showed the following symptoms after 96 h: remained for unusually long periods on the bottom of the aquarium; laid on their sides or backs; showed laboured respiration; turned dark in coloration; showed loss of equilibrium with lateral deviation from their normal orientation.

Cumulative mortality and behavioural observations (with a total number of 10 fish tested at each concentration)

Mean measured conc. [mg a.s./L]	4 h		24 h		48 h		72 h		96 h	
	No.	Obs.	No.	Obs.	No.	Obs.	No.	Obs.	No.	Obs.
Control	10	N	10	N	10	N	10	N	10	N
Solvent control	10	N	10	N	10	N	10	N	10	N
1.02	10	N	10	N	10	N	10	N	10	N
1.71	10	N	10	N	0 3 1 6	TF BO,AT,OM BO,OM,AP N	0 3 7 0	TF TS,DF,AT BO,AP,SR N	0 7 3 0	TF BO,SR, AT,DF TS,DF N
3.65	10	N	0 6 4 0	TF TS,AT,DF BO,SR, AT,DF N	1 6 3 0	TF BO,SR, AT,KR TS N	5 1 1 3 0	TF BO,SR,KR TS,AP,KR OB,SR, AT,KR N	10	TF
8.13	10	N	10	TF	10	TF	10	TF	-	-
13.4	10	N	-	-	-	-	-	-	-	-

Abbreviations:

AP: were inactive or displayed abnormally low activity
AT: showed laboured respiration
BO: remained for unusually long periods on the bottom of the aquarium
DF: turned dark in coloration
KR: had convulsions
N: did not show any abnormal signs
OB: remained for unusually long periods at the water surface
OM: open mouth
SR: laid on their sides or backs
TF: were dead
TS: showed loss of equilibrium with lateral deviation from their normal orientation
-: no observations, all fish dead

CONCLUSION

Based on mean measured concentrations, the 96 h - LC_{50} was calculated by probit analysis to be 2.59 mg a.s./L (C.I.95%: not determined due to mathematical reasons). The overall NOEC was considered to be 1.02 mg a.s./L, the highest concentration without lethal or sublethal effects.

Report:	**KIIA 8.2.1.2/02, [redacted]; 2005**
Title:	Acute toxicity of BYI 08330 (tech.) to fish (*Lepomis macrochirus*)
	Date: 2005-01-12
Organisation:	[redacted], Germany
Report No.:	DOM 24056; M-242689-01-2
Publication:	Unpublished
Dates of experimental work:	July 12, 2004 - December 6, 2004
Guidelines:	EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985) OPPTS 850.1075 (Public Draft, 1996) Directive 92/69/EEC, C.1 (1992) OECD No. 203 (rev.1992)
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the acute toxicity of the test item to Bluegill sunfish expressed as 96 h-LC_{50} for mortality. 10 fish in each test level were exposed for 96 h under static-renewal (daily) conditions to nominal concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 mg test item/L against controls. Dissolved oxygen, water temperature and pH values were determined daily in each aquarium. As measurements show, the physical/chemical properties corresponded to the required values. Contents of BYI 08330 were analysed in all test levels every day of the exposure period, new and aged test item concentrations. Based on analytical findings, all results of this study are given as nominal concentrations.

The LC_{50} (96 h) was determined to be 2.20 mg a.s./L (C.I. was not determined due to mathematical reasons).

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014 (mix-batch)** **Tox no.: 06689-00**
Purity	**99.2%**
Stability of test compound	**Expiration date: 2004-11-04, when stored at room temperature**
Water solubility	**33.5 (pH: 4) 29.9 (pH: 7), 19.1 (pH: 9) mg / L (20°C)**
2. Vehicle and/or positive control	**Acetone was used as solvent for the test item.**
3. Test animals	
Species	**Bluegill sunfish (*Lepomis macrochirus*), mean body length 4.9 cm, mean body weight 1.7 g, biomass loading was 0.43 g fish/L test medium**
Age	**Juvenile**
Source	**[redacted], USA**

Acclimation period	**> 14 days**
Environmental conditions	
Temperature	**17.9°C to 20.5°C**
Photoperiod	**16 hours light / 8 hours dark**

B Study design and methods

1. In life dates **July 12, 2004 - July 17, 2004**

2. Experimental treatments

The Bluegill sunfish (*Lepomis macrochirus*), (lot F 2/04) were obtained from and identified by [redacted], USA. All test fish were held in culture tanks on a 16/8 hour light/dark photoperiod and observed for at least 14 days prior to testing. Less than 5% mortality was noted prior to the test initiation and all unsuitable fish (e.g. injured, deformed, etc.) were eliminated from the test prior to the assignment of test groups. There was a prophylactic treatment directly after arriving of the fish with Oxytetracyclin-Hydrochlorid (4 g/100 L water, 2 × 24 h), beginning on March 31, 2004 and finishing on April 2, 2004. The definitive test started about 3.5 month after the end of prophylactic treatment.

During the acclimation period fish were fed daily with commercial trout food (Brutfutter Ecostart 17, [redacted], Denmark). They were not fed 48 h before and during the study.

Reconstituted water was prepared by adding salt stock solutions to demineralised water (conductivity < 0.2 µS/cm) to yield defined ionic concentrations. The water was then aerated to reach the oxygen saturation point and used for the test.

Based on a range finder, the definitive test concentrations were set at nominal 0.50, 1.00, 2.00, 4.00 and 8.0 mg test item/L. Untreated water and a solvent control were run in parallel. The test media were freshly prepared every day. Acetone was used as organic solvent, not exceeding a maximum load of 200 µL in 1 L test water.

The test aquaria were made of glass with a size of 32 x 36 x 38 cm (l x d x h). The test volumes amounted to 40 L. For every test concentration one aquarium was used. At the start of the test, ten fish were randomly introduced into each aquarium.

3. Observations

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

Dissolved oxygen, water temperature and pH values were determined daily in each aquarium, water temperature was additionally measured in the control aquarium and recorded hourly with a data logger. Analytical determinations of the active ingredient concentrations were made in the test medium daily, before and after the renewal of the test concentrations.

When possible, the LC_{50} values and the 95%-confidence intervals were calculated every 24-hour using a computer program, which estimated the LC_{50} using one of three statistical techniques: moving average, binomial probability or probit analysis. The appropriate method was determined according to the data characteristics.

RESULTS AND DISCUSSION

A. Findings

The test conditions met all validity criteria, given by the mentioned guidelines.
Based on analytical determination of BYI 08330 (in water by HPLC-UV, Limit of Quantitation (LOQ) = 5 µg/L) mean measured values between 91% and 99% of nominal were found in all exposure levels over the whole testing period of 96 hours. Therefore all results are given as nominal values of the test item.
The physical/chemical properties corresponded to the required values: dissolved oxygen concentrations ranged from 96 to 105% oxygen saturation, the pH values ranged from 7.2 to 7.5 and the water temperature ranged from 17.9°C to 20.5°C in all aquaria over the whole testing period.
There were neither any sublethal effects nor any mortality in the control groups. Based on nominal concentrations of BYI 08330 (tech.), the 96 h - LC_{50} was calculated by Probit analysis to be 2.20 mg a.s./L (C.I.95%: not determined due to mathematical reasons). The NOEC was considered to be 0.50 mg test item/L, the highest concentration without sublethal effects. The maximum concentration causing no mortality within the period of the test (NOLEC) was 0.5 mg test item/L. The minimum concentration causing 100% mortality (96 h) was 4.0 mg test item/L.

Exposure period (hours)	LC_{50} [mg a.s./L]	95% C.I. [mg a.s./L]	Method of statistical calculation
24	5.69	4.44 - 7.28	Probit analysis
48	3.18	n.d.	Probit analysis
72	2.20	n.d.	Probit analysis
96	2.20	n.d.	Probit analysis

n.d., not determined due to mathematical reasons

B. Observations

There were behavioural anomalies in fish caused by the test item observed over the whole exposure period in all test levels ≥ 2.0 mg/L. At the test level with 1.0 mg/L, fish showed only transient symptoms after 48 hours with recovery after 96h (in addition to mortality).

Cumulative mortality and behavioural observations (with a total number of 10 fish tested at each concentration)

Nominal conc. [mg a.s./L]	4 h		24 h		48 h		72 h		96 h	
	No.	Obs.	No.	Obs.	No.	Obs.	No.	Obs.	No.	Obs.
Control	10	N	10	N	10	N	10	N	10	N
Solvent control	10	N	10	N	10	N	10	N	10	N
0.5	10	N	10	N	10	N	10	N	10	N
1.0	10	N	10	N	2	TF	2	TF	2	TF
					1	BO,SR,AT	8	N	8	N
					7	N				
2.0	10	N	0	TF	1	TF	1	TF	1	TF
			2	BO	2	AT	1	BO	9	N
			8	N	7	N	1	H		
							7	N		
4.0	10	N	2	TF	5	TF	10	TF	10	TF
			2	OB,AP	1	OB,TS				
			6	AP	2	BO,AT				
			0	N	2	AP				
					0	N				
8.0	10	N	8	TF	10	TF	-	-	-	-
			1	V,AP						
			1	BO,SR,KR						
			0	N						

Abbreviations:
AP: were inactive or displayed abnormally low activity
AT: showed laboured respiration
BO: remained for unusually long periods on the bottom of the aquarium
H: were hyperactive / showed exaggerated response to stimulus or disturbance
KR: had convulsions
N: did not show any abnormal signs
OB: remained for unusually long periods at the water surface
SR: laid on their sides or backs
TF: were dead
TS: showed loss of equilibrium with lateral deviation from their normal orientation
V: turned in a vertical position
SD: displayed mucous excretions from the intestine
- no observations, all fish dead

CONCLUSION

Based on nominal concentrations of spirotetramat (tech.), the 96 h - LC_{50} was calculated by probit analysis to be 2.20 mg a.s./L (C.I. 95%: not determined due to mathematical reasons). The NOEC was considered to be 0.5 mg a.s./L, the highest concentration without sublethal effects.

Report:	**KIIA 8.2.1.2/03, [redacted]., [redacted] 2009**
Title:	Acute Toxicity of BYI 08330 technical to the Fathead minnow (*Pimephales promelas*) under flow through conditions Date: 2009-04-14
Organisation:	Bayer CropScience, Stilwell, Kansas, USA
Report No.:	EBFNP143; M-346157-01-1
Publication:	Unpublished
Dates of experimental work:	November 17, 2008 – November 21, 2008
Guidelines:	EPA-FIFRA § 72-1 (1982), OPPTS 850.1075 (1996) OECD 203 (1992
Deviations:	None
GLP:	yes

Executive summary

A 96-hour flow-through test was conducted to determine the acute toxicity of BYI 08330 technical to Fathead minnow (*Pimephales promelas*). The primary endpoint for acute toxicity was mortality. Sublethal and behavioral effects were also assessed during the course of the study. The test organisms were exposed under flow-through conditions to determine the 96-hour LC_{50}. The No Observed Effect Concentration (NOEC), the No Lethal Effect Concentration (NOLEC) and the Lowest Observed Effect Concentration (LOEC) were estimated. The following nominal (mean measured) concentrations were included in the study: control, solvent control, 0.50 (0.61), 1.0 (0.95), 2.0 (1.84), 4.0 (3.47) and 8.0 (7.42) mg a.s./L (ppm). There was one replicate of 10 fish each in the controls and the toxicant levels. The study met all validity criteria for an acute toxicity test acc. To the given guidelines with a mortality rate in the control(s) below 10 percent at test end and a dissolved oxygen content of greater than 60% saturation.

Mean measured recoveries ranged from 87 to 121% of nominal values. Results are based on mean measured test concentrations.

The 96h-LC_{50} based on mortality and sublethal effects was determined to be 2.84 mg a.s./L.

Based on the pattern of the 96-hour mortality data across the range of concentrations tested, the slope of the dose response curve could not be calculated.

MATERIAL AND METHODS

A Materials

1. Test material	**BYI 08330 technical**
Description	**Light beige powder**
Lot/batch No.	**Batch no.: 08045/0014 (mix-batch); TOX 07425-03**
Purity	**98.1%**
Stability of test compound	**Expiration date: 2010-04-24; storage at ambient temperature**
Water solubility:	**Test item soluble at 10 mg a.s./L, but not stable**
2. Vehicle and/or positive control	**Acetone was used as solvent for the test item.**
3. Test animals	

Species	Fathead minnow (*Pimephales promelas*), mean body length 35.0 cm, mean body weight 0.35 g, biomass loading: 0.19 g/L (mean wet weight based on controls); 0.032 g/L/day flow-through (mean wet weight based on controls)
Age	Juvenile
Source	[redacted] USA
Acclimation period	> 14 days
Environmental conditions	
Temperature	21.3°C to 22.7°C
Photoperiod	16 hours light / 8 hours dark

B Study design and methods

1. In life dates

November 17, 2008 - November 21, 2008

2. Experimental treatments

Juvenile Fathead minnow (*Pimephales promelas*) were obtained from and identified by [redacted]. All test fish were held in glass vessels on a 16/8 hour light/dark photoperiod at a temperature of 21.2-22.4°C for at least 14 days prior to testing. No mortality was noted prior to the test initiation. There were no treatments of the fish necessary before and during testing. Fish were fed daily with Tetramin flake food and live brine shrimp during the acclimation period. They were not fed 48 h before and during the study.

Stock solutions were prepared as needed. The appropriate amount of compound was weighed into a volumetric flask (corrected for percent a.i.). The flask was brought to volume with Acetone and mixed via sonicating/inverting the vessel. The stock solution was used for the diluter system on the same day it was prepared.

The test was conducted under flow-through conditions (approximately 6 volume turnover/vessel/24 hours). Nominal test concentrations were set at 0.50, 1.0, 2.0, 4.0 and 8.0 mg test item/L based upon historical toxicity data of the test item to fish. Untreated water and a solvent control (acetone) were run in parallel. Acetone was used as organic solvent, not exceeding a maximum load of 0.1 mL in 1 L test water. The test vessels were made of glass with a size of 34.3 x 21.6 x 30.5 cm (l x d x h). The test volume amounted to 18 L. For every test concentration one vessel was used. At the start of the test, ten fish were randomly introduced into each vessel. Test vessels were randomly positioned by level in the water bath.

10 fish per replicate, one replicate per level were used in the test. The control and treatment level organisms were held under similar test conditions and underwent identical procedures, except for the lack of exposure to the test compound in the case of the controls.

3. Observations

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

Dissolved oxygen, water temperature and pH values were determined daily in each aquarium, water temperature was additionally measured in the control aquarium and recorded hourly with a data logger. Analytical determinations of the active ingredient concentrations were made in the test medium at test start and after finalisation of the test (0 and 96h) from each test level.
When possible, the LC_{50} values and the 95%-confidence intervals were calculated using a computer program, which estimated the LC_{50} using one of three statistical techniques: moving average, binomial probability or probit analysis. The appropriate method was determined according to the data characteristics.

RESULTS AND DISCUSSION

A. Findings

Validity criteria given by the mentioned guidelines were met: the mortality in the control(s) did not exceed 10 percent at the end of the test, the dissolved oxygen (DO) remained ≥ 60 percent saturation.

Based on analytical determination of BYI 08330 (in water by LC/MS/MS, Limit of Quantification (LOQ): 0.05 mg a.s./L) mean measured values between 87% and 121% of nominal were found in all exposure levels over the whole testing period of 96 hours. The results of this study are given as mean measured concentrations, equivalent to 0.61, 0.95, 1.84, 3.47 and 7.42 mg a.s./L.
The physical/chemical properties corresponded to the required values: dissolved oxygen concentrations ranged from 83 to 93% oxygen saturation, the pH values ranged from 7.9 to 8.1 and the water temperature ranged from 21.3°C to 22.7°C over the whole testing period.
There were neither sublethal effects nor any mortality in the control groups. Based upon mean measured concentrations, the 96 h - LC_{50} was calculated by binomial probability to be 2.84 mg a.s./L (C.I.95%: 1.84-7.42). The overall NOEC was found to be 0.95 mg a.s./L, the highest concentration without lethal or sublethal effects. The maximum concentration at which no mortality was observed after 96 hours (NOLEC) was 1.84 mg a.s./L.

Calculated LC_{50} values

Exposure period (hours)	LC_{50} [mg a.s./L]	95% C.I. [mg a.s./L]	Method of statistical calculation
24	5.84	> 3.47	Binomial probability
48	4.68	3.47 – 7.42	Binomial probability
72	3.20	1.84 – 7.42	Binomial probability
96	2.84	1.84 – 7.42	Binomial probability

Acute toxicity to Fathead minnow

Test Substance	BYI 08330 Technical
Test Object	Fathead minnow
Exposure	96-Hour, Flow-through

LC50 96 hours (95% C.I.)	2.84 (1.84 and 7.42) mg a.s./L
Lowest Concentration With an Effect (LOEC)	1.84 mg a.s./L
Highest Concentration Without Toxic Effect (NOEC)	0.95 mg a.s./L
Highest Concentration Causing No Mortality (NOLEC)	1.84 mg a.s./L

The 96-hour LC_{50} was calculated by binomial probability. The method selected was determined by the characteristics of the data, i.e., the number of concentrations in which mortalities between 0 and 100% occurred and the 95% confidence intervals ([redacted], 1977/1984). The software was unable to calculate the LC_{50} using the probit method and associated slope of the dose response curve. The NOEC and LOEC were empirically determined based upon observational data including sublethal effects.

B. Observations

No behavioural abnormalities were found in the 0.61 and 0.95 mg a.s./L treatment levels and in both controls. There were behavioural anomalies in fish observed starting 24 hours after test start in the highest treatment level of 7.42 mg a.s./L (mean measured). After 48 and 72 hours, anomalies were found in the 3.47 mg a.s./L treatment group. At study end, effects were also noted for the 1.84 mg a.s./L treatment group (symptoms see table below).

Cumulative mortality and behavioural observations of the Fathead minnow exposed to BYI 08330 technical

Mean Measured Concentration	Hour 4		24 Hour		48 Hour		72 Hour		96 Hour	
(mg a.s./L)	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
Control	0	10 N	0	10 N	0	10 N	0	10 N	0	10 N
Solvent Control	0	10 N	0	10 N	0	10 N	0	10 N	0	10 N
0.61	0	10 N	0	10 N	0	10 N	0	10 N	0	10 N
0.95	0	10 N	0	10 N	0	10 N	0	10 N	0	10 N
1.84	0	10 N	0	10 N	0	10 N	0	10 N	0	9 N; 1 LE, OB, Q
3.47	0	10 N	0	10 N	1	7 N; 2 LE; 1 D	6	3 N; 1 LE; 5 D	8	1 N; 1 E, LE; 2 D
7.42	0	10 N	0	2 OB, Q, LR; 8 D	10	2 D	---	---	---	---

Obs = Observations (number of individuals observed plus observation)
LE = Loss of Equilibrium, Q = Quiescent
Dead = Cumulative number of dead
OB = On Bottom, E = Erratic Behavior
D = Dead, N = Normal, LR = Labored Respiration
--- = No observations taken

Note: There were 10 organisms present in each test concentration at the start of the test

Mean Measured Test Concentration (mg a.s./L)	Sublethal Observations (number with sublethal effects/number of survivors)				
	Hour 4	24 Hour	48 Hour	72 Hour	96 Hour
Control	0/10	0/10	0/10	0/10	0/10
Solvent Control	0/10	0/10	0/10	0/10	0/10
0.61	0/10	0/10	0/10	0/10	0/10
0.95	0/10	0/10	0/10	0/10	0/10
1.84	0/10	0/10	0/10	0/10	1/10
3.47	0/10	0/10	2/9	1/4	1/2
7.42	0/10	2/2	0/0	0/0	0/0

There were no mortalities in the treatment group of 1.84 mg a.s./L and 100% mortality at 7.42 mg a.s./L (mean measured).

Mortality	
Mean Measured Test Concentration (mg a.s./L)	Percent Dead at 96-Hours
Control	0%
Solvent Control	0%
0.61	0%
0.95	0%
1.84	0%
3.47	80%
7.42	100%

CONCLUSION

The 96h-LC50 (95% C.I.) was determined to be 2.84 mg a.s./L (1.84 - 7.42 mg a.s./L). The NOEC and LOEC were empirically determined based upon observational data including sublethal effects. The 96h-LOEC and NOEC were determined to be 1.84 mg a.s./L and 0.95 mg a.s./L, respectively. The NOLEC was at 1.84 mg a.s./L.

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

Report:	**KIIA 8.2.1.3/01, [redacted]; 2005**
Title:	Acute toxicity of BYI 08330-enol to fish (*Oncorhynchus mykiss*) Date: 2005-05-18
Organisation:	[redacted], Germany
Report No.:	EBFNM013; M-251850-01-2
Publication:	Unpublished
Dates of experimental work:	December 06, 2004 - December 15, 2004
Guidelines:	EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985) OPPTS 850.1075 (Public Draft, 1996) Directive 92/69/EEC, C.1 (1992) OECD No. 203 (rev. 1992)
Deviations:	no major deviations
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the acute toxicity of the test item to Rainbow trout expressed as 96 h-LC_{50} for mortality. Thirty fish (fifteen per aquarium) were exposed in a limit test for 96 h under static test conditions to a nominal concentration of 100.9 (100) mg test item (pure metabolite)/L against two water control groups (each with 15 fish). Dissolved oxygen, water temperature and pH values were determined daily in each aquarium. As measurements show, the physical/chemical properties corresponded to the required values. Recoveries of BYI 08330-enol were measured in all test levels on day 0, day 2 and day 4 of the exposure period. Due to analytical findings all results are given as nominal concentration of the pure metabolite The LC_{50} (96 h) of BYI 08330-enol was determined to be > 100 mg pure metabolite/L.

MATERIAL AND METHODS

A Materials

1. Test material	**BYI 08330-enol**
Description	**Beige powder**
Lot/batch No.	**Batch no.: 692-101-09-0005** **Tox no.: 06850-00**
Purity	**99.1%**
Stability of test compound	**Expiration date: 2005-03-17 when stored at room temperature**
Water solubility	**1.751 g/L (pH 7)**
2. Vehicle and/or positive control	**-**
3. Test animals	
Species	**Rainbow trout (*Oncorhynchus mykiss*), mean body length 4.9 cm, mean body weight 1.3 g, biomass loading was 0.49 g fish/L test medium**
Age	**Juvenile**
Source	**[redacted] Germany.**
Acclimation period	**> 14 days**

Environmental conditions

Temperature	**11.1°C to 13°C**
Photoperiod	**16 hours light / 8 hours dark**

B Study design and methods

1. In life dates **December 06, 2004 – December 10, 2004**

2. Experimental treatments

Rainbow trout (*Oncorhynchus mykiss*), (lot F 9/04) were obtained from and identified by from [redacted], Germany. The parent fish were identified to species by the supplier. They hatched in the testing facility. All test fish were held in culture tanks on a 16/8 hour light/dark photoperiod and were observed for at least 14 days prior to testing. Mortality was noted prior to the test initiation and all unsuitable fish (e.g. injured, deformed, etc.) were eliminated from the test prior to the assignment of test groups. In the 48 hour acclimation period before testing less than 5 percent of the fish died. There was no treatment of the fish necessary before and during testing.

Fish were fed daily with commercial trout food (Brutfutter Ecostart 17, [redacted], Denmark) during the acclimation period. They were not fed 48 h before and during the study.

Reconstituted water was prepared by adding salt stock solutions to demineralised water (conductivity < 0.2 µS/cm) to yield defined ionic concentrations. The water was then aerated to reach the oxygen saturation point and used for the test.

Based on a range finder test, the definitive test concentration was set at 100 mg pure metabolite/L (nominal). Two untreated water controls were run in parallel. The test media of the two treated aquaria (A & B) was prepared by mixing the test item into 4 L of test water as homogeneously as possible by intense stirring and heating up to 30°C. The pH was adjusted to about 7 with NaOH. Then the mixture was transferred quantitatively into the aquarium and filled up to 40 L. The test aquaria were made of glass with a size of 32 x 36 x 38 cm (l x d x h). For every test concentration one aquarium was used. At the start of the test, fifteen fish were randomly introduced into each aquarium.

3. Observations

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

Dissolved oxygen, water temperature and pH values were determined daily in each aquarium, water temperature was additionally measured in the control aquarium and recorded hourly with a data logger. Analytical determinations of the active ingredient concentrations were made in the test medium daily, before and after the renewal of the test concentrations.

When possible, the LC_{50} values and the 95%-confidence intervals were calculated every 24-hour using a computer program, which estimated the LC_{50} using one of three statistical techniques: moving average, binomial probability or Probit

analysis. The appropriate method was determined according to the data characteristics.

RESULTS AND DISCUSSION

A. Findings

Test conditions met all validity criteria given by the mentioned guidelines. There was a temperature deviation of more than 1°C in the treated aquaria (1.5°C: replicate A; 1.3°C: replicate B) within the first 24 hours caused by test item preparation. This minor deviation did not influence the results of the study.

The analytical findings reflect the expected nominal concentration of the test item. The test item was stable under conditions of testing. Based on analytical determination of BYI 08330-enol (in water by HPLC - UV, LOQ = 5 µg/L) mean measured values of 99% (replicate A) and 98% (replicate B) of nominal were found over the whole testing period of 96 hours. Therefore all results are given as nominal concentration of the pure metabolite.

The physical/chemical properties corresponded to the required values: dissolved oxygen concentrations ranged from 86 to 102% oxygen saturation, the pH-values ranged from 6.7 to 7.2 and the water temperature ranged from 11.1°C to 13.0°C in all aquaria over the whole testing period. The minimum concentration causing 100% mortality after 96h was > 100 mg pure metabolite/L.

There were neither sublethal effects nor any mortality in the control group. The 96h-LC_{50} of BYI 08330-enol was > 100 mg p.m./L. No mortality was noted in the study after 96 hours. The concentration required to cause 100% mortality after 96 h is estimated to be > 100 mg p.m./L. The no-observed-lethal-effect concentration (NOLEC) after 96 h was ≥ 100 mg p.m./L.

B. Observations

Test item was observed at the surface at beginning of the test. After 24, 48 and 72 hours, test item was found lying at the bottom of the aquaria; after 96 h precipitation of the test item was observed.

There were neither any sublethal effects nor any mortality in any of the tested groups.

Cumulative mortality and behavioural observations (with a total number of 30 fish (15 fish per replicate) tested in each test level)

Exposure time	4 h		24 h		48 h		72 h		96 h	
Test level	no. of dead	Obs.	no. of dead	Obs.	no. of dead	Obs.	no. of dead	Obs.	no. of dead	Obs.
Control A	0	15 N	0	15 N	0	15 N	0	15 N	0	15 N
Control B	0	15 N	0	15 N	0	15 N	0	15 N	0	15 N
100 mg p.m./L A	0	15 N	0	15 N	0	15 N	0	15 N	0	15 N
100 mg p.m./L B	0	15 N	0	15 N	0	15 N	0	15 N	0	15 N

p.m. pure metabolite
N: did not show any abnormal signs

CONCLUSION

The 96 h-LC_{50} of BYI 08330-enol to Rainbow trout (*Oncorhynchus mykiss*) under static test conditions was > 100 mg p.m./L.

There were neither behavioural abnormalities nor mortality of fish caused by the test item over the whole exposure period. Therefore the no-observed-effect-concentration (NOEC) after 96 h was ≥ 100 mg p.m./L.

Report:	**KIIA 8.2.1.3/02, [redacted]; 2006**
Title:	4-Methoxycyclohexanone; Acute fish toxicity Date: 2006-07-12
Organisation:	Bayer Industry Services, Leverkusen, Germany
Report No.:	2006/0032/02; M-274305-01-1
Publication:	Unpublished
Dates of experimental work:	June 16, 2006 – July 10, 2006
Guidelines:	Directive 92/69/EEC, C.1 (1992), in most parts equivalent to OECD No. 203 (rev.1992)
Deviations:	Some test fish had a somewhat larger size than recommended in the test guideline for zebra fish. This deviation is not regarded to be relevant to the results.
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the acute toxicity of 4-Methoxycyclohexanone to Zebra fish expressed as 96 h-LC50 for mortality. 10 fish were exposed for 96 h under static conditions (without renewal of medium) in a limit test to a nominal concentration of 100 mg p.m./L against an untreated control. Dissolved oxygen, water temperature and pH values were determined daily in each aquarium. As measurements show, the physical/chemical properties corresponded to the required values. 4-Methoxycyclohexanone contents were analysed at test start and after 24 and 96 hours of the exposure period. Since recoveries were between 89.7% and 104.9% of nominal, the results of this study are given as nominal concentrations.
The LC_{50} (96 h) was determined to be > 100 mg p.m./L, as no toxic effects against fish were observed at a concentration of 100 mg p.m./L.

MATERIAL AND METHODS

A Materials

1. Test material	**4-Methoxycyclohexanone**
Description	**Not stated**
Lot/batch No.	**Batch no.: SAV5506-001**
Purity	**97.9%**
Stability of test compound	**Not stated**
Water solubility	**Poorly soluble (according to data of the sponsor)**
2. Vehicle and/or positive control	**No vehicle and/or positive control**
3. Test animals	
Species	**Zebra fish (*Danio rerio*), mean body length 3.4 cm (SD 0.26), biomass loading was 0.64 g fish (w.w.)/L test medium**
Age	**Not stated**
Source	**[redacted] Germany**

Acclimation period	**Stock held since 2006-03-03 and acclimatised to the test conditions since then**
Environmental conditions	
Temperature	**20 - 24°C (mean +/- 1°C)**
Photoperiod	**16 hours light / 8 hours dark**

B Study design and methods

1. In life dates **June 16, 2006 – July 10, 2006**

2. Experimental treatments

Zebra fish (*Danio rerio*) were obtained from [redacted], Germany, on 2006-03-03. The stock was held and acclimatised to the test conditions since then (20 – 24°C, > 5 mg/L dissolved oxygen, 16/8 hour light/dark photoperiod). Mortality was evaluated prior to the test initiation. In the acclimatisation period before testing less than 5% of the fish died. There was no treatment of the fish necessary before and during testing.

Fish were fed daily with commercial fish food during the acclimation period. They were not fed 24 h before and during the study.

Reconstituted water was prepared according to the recommendations of ISO 7346 (total hardness at test start 13.8 °dH, corresponding to 246.33 mg/L $CaCO_3$). The water was used for both the maintenance of the test animals under flow-through conditions and the preparation of stock and test solutions of the test item.

The limit test concentration was 100 mg p.m./L (nominal). To achieve this concentration, the respective amount of the test item was added to 5 L of dilution water and treated with an ultra turrax for 60 sec at 8000 rpm. Untreated water (control) was run in parallel. The test medium was not renewed during the test.

The test aquaria were made of glass, and the test volumes amounted to 5 L. For every test treatment, one aquarium was used. At the start of the test, ten fish were introduced into each aquarium containing the prepared media. Test vessels were gently aerated via narrow glass tubes.

3. Observations

During the test, fish were examined after two hours and then daily for mortalities and signs of poisoning (type and incidence of sub-lethal effects) by visual observations.

Dissolved oxygen, water temperature and pH values were determined daily in each aquarium. Analytical determinations of the test concentrations in the test medium were made at test start and after 24 and 96 hours in the test medium.

When possible, the LC_{50} values were determined every 24 hours by Probit analysis.

RESULTS AND DISCUSSION

A. Findings

The test conditions met all validity criteria given by the mentioned guidelines.

Based on analytical determination of 4-Methoxycyclohexanone (by GC-MS), recovery rates corresponded to 104.9% of nominal value at 0 hours, to 100.7% of nominal value at 24 hours and to 89.8% of nominal value at 96 hours.
The physical/chemical properties corresponded to the required values: dissolved oxygen concentrations ranged from 88 to 95% oxygen saturation, the pH values ranged from 7.6 to 7.9 and the water temperature ranged from 20.6°C to 21.0°C in the aquaria over the whole testing period
There were neither any sublethal effects nor any mortality in the control groups. Based on nominal concentrations of 4-Methoxycyclohexanone, the 96 h - LC_{50} was determined by Probit analysis to be > 100 mg p.m./L.

Exposure period (hours)	LC_{50} [mg p.m./L]	Method of statistical calculation
2	> 100	Probit analysis
24	> 100	Probit analysis
48	> 100	Probit analysis
72	> 100	Probit analysis
96	> 100	Probit analysis

B. Observations

There were behavioural anomalies (abnormal swimming action) in fish caused by the test item only at the 2 hour observation period at the test level of 100 mg p.m./L.

Cumulative mortality and behavioural observations (with a total number of 10 fish tested in each treatment)

Nominal conc. [mg p.m./L]	2 h		24 h		48 h		72 h		96 h	
	No.	Obs.	No.	Obs.	No.	Obs.	No.	Obs.	No.	Obs.
Control	10	N	10	N	10	N	10	N	10	N
100	5	N	10	N	10	N	10	N	10	N
	5	AS								

Abbreviations:
N: did not show any abnormal signs
AS: abnormal swimming action

CONCLUSION

Based on nominal concentrations of 4-Methoxycyclohexanone, the 96 h - LC_{50} was determined by Probit analysis to be > 100 mg p.m./L.

IIA 8.2.2 Chronic toxicity to fish

Chronic toxicity to fish is covered by studies presented in IIA 8.2.3 to IIA 8.2.5.

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

The chronic toxicity test to juvenile fish is covered by the fish early life stage limit test (see IIA 8.2.4/01).

IIA 8.2.4 Fish early life stage toxicity test

Report:	**KIIA 8.2.4/01, [redacted]; 2005**
Title:	Early-life Stage Toxicity of BYI 08330 tech. to Fish (*Pimephales promelas*). Date: 2005-11-16
Organisation:	[redacted], Germany
Report No.:	EBFN0305; M-206067-01-2
Publication:	Unpublished
Dates of experimental work:	March 24, 2005 - May 11, 2005
Guidelines:	EPA-FIFRA § 72-4a/SEP-EPA-560/6-82-002 (1982) ASTME 1241-92 (1992) OPPTS 850.1400 (1996) OECD No. 210(1992)
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the toxicity of the test item during the early life stages of Fathead minnow (*Pimephales promelas*), expressed as NOEC, LOEC and MATC. Early-life stages of fathead minnow (eggs, larvae and fry) were exposed to various test item concentrations, a control and a solvent control under continuous flow conditions with four replicates per test concentration and controls for 33 days (28 days post-hatch). The definitive study was conducted at the nominal test concentrations of 0.0625 (0.0607), 0.125 (0.121), 0.250 (0.243), 0.500 (0.486) and 1.00 (0.971) mg test item (a.s.)/L.

Dissolved oxygen, water temperature and pH values were determined and as measurements show, the results corresponded to the required values. Recoveries of BYI 08330 were measured in the test media weekly from alternating replicates on days -1, 0, 7, 14, 21, 28 and 33. Due to analytical findings, the reported results refer to the mean measured concentrations of BYI 08330 in water.

The overall chronic 33-day-NOEC for spirotetramat to early life stages of fathead minnow is 0.534 mg a.s./L (based on fry survival) and the overall 33-day-LOEC is 1.16 mg a.s./L (MATC = 0.787 mg a.s./L).

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014 (mix-batch)** **Tox no.: 6689-01**
Purity	**97.1%**
Stability of test compound	**Expiration date: 2005-05-05 when stored at room temperature**
2. Vehicle and/or positive control	**Dimethylformamide (DMF), 0.1 mL/L**
3. Test animals	

Species	**Fathead minnow (*Pimephales promelas*)**
Age	**Freshly fertilized eggs (< 24-hours old) were used at the start of exposure.**
Source	**Eggs were obtained from a brood stock cultured in the test facility at [redacted].**
Acclimation period	**The in-house culture was maintained at the actual test temperature and in water of the same quality used in the test.**
Environmental conditions	
Temperature	**23.0 to 26.0°C**
Photoperiod	**16 hour light / 8 hours dark with a 30 minutes transition period, light intensity was between 183 - 227 lux**

B Study design and methods

1. In life dates **March 24, 2005 - May 05, 2005**

2. Experimental treatments

Freshly fertilized fathead minnow eggs (< 24-hours old) of eight breeding tanks (one breeding tank = ♀+♀) were used in this study. Eggs were obtained from a brood stock cultured in the test facility at [redacted]AG. The in-house culture was maintained at the actual test temperature and in water of the same quality used in the test. Reconstituted water was prepared by adding salt stock solutions to demineralised water (conductivity < 0.2 µS/cm) to yield defined ionic concentrations. The dilution water was maintained at 24.5° ± 1.5°C with a flow-through thermostat and aerated to oxygen saturation. Eggs were incubated in incubation cups constructed from 5.5 cm diameter stainless steel pipes with a screened stainless steel bottom perforated with holes (hole diameter: 0.8 mm). The glass aquaria were approximately 12.5 cm x 14 cm with a water depth of 21.5 cm, yielding an approximate chamber volume of 3.75 litres and resulting in approximate 12 changes of test water per day.

The biological phase of the final study was initiated (study day 0) by distribution of 25 impartially selected freshly (< 24 h old) fertilized eggs into each of the egg incubation cups with a glass pipette. There were 4 replicate exposure chambers per concentration. The definitive study was conducted at the nominal test concentrations of 0.0625 (0.0607), 0.125 (0.121), 0.250 (0.243), 0.500 (0.486) and 1.00 (0.971) mg test item (a.s.)/L.

Feeding was started shortly after the larvae had hatched. They were fed live newly hatched brine shrimp nauplii (< 24h old) *ad libitum*, taking care that each aquarium receives an equal quantity of food. Food was added to the aquaria five times daily, except on weekends/holidays when food was added twice daily until one day before study termination.

3. Observations

Every day all incubation cups were observed for embryo mortality, as discerned by a distinct change in coloration. Dead embryos were recorded and discarded.
Hatched larvae were recorded and released into the test chambers by allowing them to swim out of each embryo cup into the corresponding replicate aquaria. After completion of hatch (day 5 after start of exposure, day 0 post-hatch) larvae were thinned to the desired number of 15 individuals per egg cup by impartially selection. Observations of visible abnormalities and mortalities were recorded on weekdays. Dead fry were removed and discarded.
At test termination of the biological phase (post hatch day 28) after a total of 33 days the surviving fish were sacrificed. The standard length (mm) was determined and recorded for each individual fish. Wet weight of all control fish and solvent control fish was recorded for evaluation of the system biomass loading. Once length and wet weight measurements were completed each single fish was then placed into labelled open pans and placed in a 60°C drying oven for 2 days. The dry weights of the individual fish were measured with an accuracy level of 0.1 mg using an analytic balance.
Water temperature was measured in one alternating replicate of the control, solvent control and all other test levels on study days 0, 7, 14, 21, 28 and 33. The temperature in one control aquarium was recorded hourly by a data logger.
Dissolved oxygen (in percent saturation) was measured in one alternating replicate of the control, solvent control and all other test levels on study days 0, 7, 14, 21, 28 and 33.
pH-values were measured in one alternating replicate of the control, solvent control and all other test levels on study days 0, 7, 14, 21, 28 and 33.
Total hardness, expressed in °dH (German hardness; 1°dH = 17.8 mg $CaCO_3$/L) was measured in one replicate of the control, the lowest, the middle and the highest test level on study days 0 and 33.
Conductivity (in µS/cm = 1 µmhos/cm) was measured in the dilution water before splitting and were documented hourly by a data logger.
Samples of test solutions, including controls, were taken from alternating replicate test chambers on days -1, 0, 7, 14, 21, 28 and 33 in order to measure actual test substance exposure concentrations.
Replicate means were used for statistical analysis. For each parameter analysed the following statistical tests were conducted: t-test to determine if control and solvent control data can be pooled, R/s - test procedure in order to test the correspondence with normal distribution, Cochran's test for homogeneity of variances. Control data were pooled if the t-test criteria were met.
Time to hatch, hatching success and fry survival data were arcsine transformed before analysis using the Williams-t-Test. Growth data, expressed as dry weight and as length, were analysed without previous data transformation using the Williams-t-Test.

Statistical analyses were conducted using a PC based computer program developed by ToxRat Solutions GmbH, with conclusions of statistical significance based on a 95% confidence level (a = 0.05).

RESULTS AND DISCUSSION

A. Findings

Test conditions met all validity criteria, given by the mentioned guidelines.
The analytical findings (in water by HPLC-UV, limit of Quantitation (LOQ) = 5 µg/L) reflect the expected nominal concentrations of the test item. The mean measured concentrations of spirotetramat were 0.0566, 0.115, 0.247, 0.534 and 1.16 mg a.s./L, which ranged from 93 to 119 percent of nominal during the test period for all test levels. The reported results refer to the mean measured concentrations of BYI 08330 in water.

Water temperature measured continuously in a control aquarium ranged from 23.0 to 26.0°C. Water temperature measured once a week (single point measurements in all test levels) ranged from 24.0 to 25.0°C. Dissolved oxygen (DO) concentrations ranged from 85 to 100 percent oxygen saturation and pH values ranged from 7.0 to 7.3 in all aquaria over the whole period of testing. Total hardness ranged from 2.5 to 2.9 °dH in the control, the lowest, the middle and the highest test level during the total test period. Conductivity, measured in dilution water, ranged in the hourly mean values from 96 to 140 µS/cm. Light intensity in the room was between 183 - 227 lux with a photoperiod of 16 hours.
The biomass loading factor for the study was determined using the wet weights of the control and solvent control fish at study termination. The mean wet weight was 112 mg. The biomass loading factor based upon the 3.75 litre volume of a single growth chamber was 0.4 g/L. The biomass loading factor based on a flow of 45 litres per day through each single test chamber was 0.04 g/L/day. These values were well within the requirements to ensure adequate dissolved oxygen levels and to avoid fish crowding.

Hatching Success

Percent egg hatchability was evaluated on day 9 post hatch. Hatch data ranged from 84% to 91% for all test levels and the controls. There was no significant difference in hatching success in any test level compared to the pooled control data.

Egg hatching began on study day 4 and continued until study day 5 (day 0 post hatch) in all test levels and in the controls. Hatching success was evaluated on day 0 post hatch:

Mean measured conc. [mg a.s./L]		phd -1* (Study day 4) Egg Hatch [%]	Mean measured conc. (mg a.s./L)		phd 0 (Study day 5) Egg Hatch [%]
Solvent Control	Mean (SD)	61 (11.9)	Pooled Controls	Mean (SD)	85 (8.8)
0.0566	Mean (SD)	47 (29.6)	0.0566	Mean (SD)	85 (11.5)
0.115	Mean (SD)	52 (10.8)	0.115	Mean (SD)	84 (8.0)
0.247	Mean (SD)	40 (11.8)	0.247	Mean (SD)	88 (5.7)
0.534	Mean (SD)	67 (7.6)	0.534	Mean (SD)	91 (5.0)
1.16	Mean (SD)	48 (12.6)	1.16	Mean (SD)	84 (8.0)

SD Standard Deviation phd post hatch day.
*On post hatch day -1 the water control was excluded from statistical data analysis because water control and solvent control could not be pooled.

Fry survival

Fry survival was analysed at test termination as overall fry survival (sum of fry survival before and after thinning). Overall fry survival ranged from 65 to 87 percent: Control (87%), Solvent Control (86%), 0.0566 mg a.s./L (82%), 0.115 mg a.s./L (81%), 0.247 mg a.s./L (85%), 0.534 mg a.s./L (73%) and 1.16 mg a.s./L (65%). Data analysis showed statistically significant differences in comparison to the pooled control data in the test level at 1.16 mg a.s./L.

Fry survival was analysed at test termination as overall fry survival (fry survival before and after thinning):

Mean measured conc. [mg a.s./L]		Fry Survival (study day 5/ phd 0) [%]	Fry survival (study day 33/phd 28) [%]	Overall Fry Survival** [%]
Pooled Controls	Mean (SD)	98 (2.6)	88 (11.1)	87 (11.1)
0.0566	Mean (SD)	94 (9.0)	87 (16.3)	82 (18.4)
0.115	Mean (SD)	95 (5.9)	85 (6.4)	81 (6.0)
0.247	Mean (SD)	100 (0)	85 (17.5)	85 (17.5)
0.534	Mean (SD)	92 (4.7)	78 (19.9)	73 (21.7)
1.16	Mean (SD)	83 (7.1)	78 (14.8)	65 (11.5)*

* Denotes statistically significant difference from pooled controls (α=0.05, Williams Test)
** (fry survival before thinning/number of fry hatched)×(fry survival/number of fry after thinning)×100

Fry growth

Fry growth, expressed as length and dry weight, was measured at test termination (study day 33 / post hatch day 28). Data analysis did not show statistically significant difference in comparison to the pooled control data.

Fry growth was analysed at test termination (study day 33 / post hatch day 28):

Mean measured conc. (mg/L)		Study Day 33 (phd 28) Length [mm]	Dry weight [mg]
Pooled Controls	Mean (SD)	18.0 (3.5)	20.4 (7.4)
0.0566	Mean (SD)	18.4 (3.4)	21.8 (10.4)
0.115	Mean (SD)	18.3 (3.4)	20.4 (11.3)
0.247	Mean (SD)	18.3 (3.9)	21.5 (11.4)
0.534	Mean (SD)	19.1 (2.2)	22.1 (7.5)
1.16	Mean (SD)	18.6 (3.4)	21.9 (10.4)

B. Observations

There were no morphological and behavioural effects observed.

CONCLUSION

Based on the observations and statistical analysis (Williams-t-test) of time to hatch, hatching success, fry survival, fry growth (expressed as weight and length) and morphological and behavioural effects, the test revealed the following results (based on mean measured concentrations of BYI 08330 in water):

Time to hatch (study day 4-5)	NOEC	≥ 1.16 mg a.s./L	LOEC	> 1.16 mg a.s./L
Hatching success (study day 5)	NOEC	≥ 1.16 mg a.s./L	LOEC	> 1.16 mg a.s./L
Fry survival (study day 33)	NOEC	0.534 mg a.s./L	LOEC	1.16 mg a.s./L
Length (study day 33)	NOEC	≥ 1.16 mg a.s./L	LOEC	> 1.16 mg a.s./L
Weight (study day 33)	NOEC	≥ 1.16 mg a.s./L	LOEC	> 1.16 mg a.s./L
Morphological & Behavioural Effects	NOEC	≥ 1.16 mg a.s./L	LOEC	> 1.16 mg a.s./L

The overall chronic 33-day-NOEC for spirotetramat to early life stages of Fathead minnow is 0.534 mg a.s./L (based on fry survival) and the overall 33-day-LOEC is 1.16 mg a.s./L (MATC = 0.787 mg a.s./L).

IIA 8.2.5 Fish life cycle test

A fish life cycle test is not required for spirotetramat as the substance is not persistent in water (DT_{90} < 10 d) and no potential for bioaccumulation is indicated (see KIIA 8.2.6.1).

IIA 8.2.6 Bioconcentration potential in fish

IIA 8.2.6.1 Bioconcentration potential of the active substance in fish

Since the octanol/water partition coefficient (log Pow) of spirotetramat is < 3 (2.51, see KIIA 2.8.1), a potential of bioconcentration is not indicated and thus, a study is not required.

IIA 8.2.6.2 Bioconcentration potential of the metabolites, degr. & react. products

Due to the low log P_{OW} of the spirotetramat metabolites, the potential of bioaccumulation can be considered negligible (the log P_{OW} of BYI 08330-cis-ketohydroxy is 1.3 and of BYI 08330-enol is 0.3 (at pH 7), see KIIA 7.13) and thus, a study is not deemed necessary.

IIA 8.2.7 Aquatic bioavailability/ biomagnification / depuration*

Due to the low lipophilicity of spirotetramat, no specific studies on bioconcentration were performed and no specific concerns regarding bioavailability, biomagnification and depuration have to be addressed.

*** No EC data requirement (the OECD point concerned is not covered by or part of an EC point according to Council Directive 91/414/EEC. Hence, data/documents do not need to be submitted.**

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

IIA 8.3.1 Acute toxicity to aquatic invertebrates

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

Report:	**KIIA 8.3.1.1/01, [redacted] 2005**
Title:	Acute Toxicity of BYI 08330 (tech.) to the Waterflea *Daphnia magna* under static conditions Date: 2005-01-14
Organisation:	[redacted], Germany
Report No.:	DOM 24004; M-242683-01-2
Publication:	Unpublished
Dates of experimental work:	July 12, 2004 – October 22, 2004
Guidelines:	OECD – 202,April 4, 1984 and corresponding revised draft proposal, dated February 01, 2004; EEC Directive 92/69/EWG, part C.2; U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72 2, OPPTS Guideline 850.1010 public draft 1996 (modified); JMAFF 12 Nousan No. 8147 (2000)
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the influence of the test item on mobility of *Daphnia magna* over 48 hours in a static exposure, expressed as EC_{50} for immobilisation. *Daphnia magna* (< 24 h old, 6 x 5 animals per concentration) were exposed in a static test system for 48 hours to nominal concentrations of 0, 6.25, 12.5, 25, 50 and 100 mg a.s./L without feeding (corresponding to mean-measured concentrations of 4.70, 8.77, 20.3, 38.8 and 42.7 mg a.s./L). As measurement show, the physical / chemical properties of the test water (pH, temperature, oxygen-saturation, hardness) corresponded to the aspired values. Due to analytical findings all results are given as mean measured test concentrations of the active substance.
Only 23% immobility occurred after 48 hours of static exposure at the maximum exposure concentration and the EC_{50} for spirotetramat is thus determined to be greater than 42.7 mg a.s./L.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014 (mix-batch)** **Tox no.: TOX 06689-00**
Purity	**97.2%**
Stability of test compound	**Sample released until 2004-11-04**

2. Vehicle and/or positive control	**Test and breeding water was prepared as "ELENDT M7 medium".** **Acetone was used as solvent for the test item.**
3. Test animals	
Species	***Daphnia magna***
Age	**1st instars (< 24 h old)**
Source	**Strain from the [redacted], Germany, from laboratory stock breeding. This breeding strain has been maintained in the test facility successfully for more than fifteen years in weekly renewed aqueous media.**
Acclimation period	**The first instars used in the test are obtained by repeated careful screening of an in-house breeding-culture of defined age less than 24h before test initiation. They descend from at least the fourth (or later) brood of parent daphnids (21 days ± 12 hours old). No males, ephippia or dead animals were present in the cultures. The cultures showed no delay in first brood.**
Environmental conditions	
Temperature	**20 ± 1°C**
Photoperiod	**16:8 hours, approx. 1500 Lux**

B Study design and methods

1. In life dates **July 12, 2004 - July 15, 2004**

2. Experimental treatments

***Daphnia magna* are kept in 2 L glass-containers (50 to 100 daphnids per container) in a climate controlled environment at 20 ± 1°C and a 16 : 8 hour light-dark cycle. They were fed three times per week with the single cell green algae *Desmodesmus subspicatus*, supplemented by a commercial fish food (trade name: TetraMin®) in aqueous suspension.**

Based on the results of an range finding test, the actual study included six geometrically spaced nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg a.s./L (spacing factor 2.0). An untreated dilution water (blank) control and an additional solvent control (0.4 mL acetone/L) were exposed under identical conditions. Since hydrolysis stability data for BYI 08330 are not suitable to guarantee sufficient exposure over 48 hours under natural pH of the test media (pH 8), the pH of the test water was adjusted to pH 6 before use for dilution procedure. Preparation of test solutions occurred immediately before the start of exposure.

The test vessels consisted of 100 mL glass beakers, filled with 50 mL of the test solution. Six vessels (replicates), each provided with five daphnids (= 10 mL test solution per daphnid), were used per treatment group and controls (=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. 1500 lux. The waterfleas were exposed to BYI 08330 (tech.) over a 48 hours period under static conditions.

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

3. Observations

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 possible signs of sublethal effects had to be recorded.

Prior to each preparation of test concentrations, conductivity, total hardness and alkalinity of the dilution media (ELENDT M7) were measured. The dissolved oxygen and pH values were measured in the freshly prepared test solutions of each treatment level and control and in the pooled replicates of the aged media at test termination (day 2). Light intensity was measured at start of the study with a photometer. Environmental temperature was recorded continuously with a thermohygrometer. 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 the study.

For analytical verification of the test item concentrations, duplicate samples of the freshly prepared test media have been taken on day 0 from all test levels and the controls prior to introducing the test animals. For additional stability measurements the aged replicates of each remaining concentration level were pooled after 48 hours of exposure and samples were then taken from this pooled media at study termination. The limit of quantification (LOQ) for the method used (in water by HPLC-UV) was 5 µg/L. The method was validated within the current study.

RESULTS AND DISCUSSION

A. Findings

No immobilisation of untreated control animals occurred. Sensitivity of the used daphnid breeding-strain is located within the required range as verified by periodically performed reference substance testing. Thus, the study conditions and breeding quality met the required quality criteria.

The measured concentrations of spirotetramat in the freshly prepared test solutions at test initiation ranged between 63% and 90% of the corresponding nominal concentrations. The measured concentrations of the aged test solutions at the end of exposure ranged between 22% to 81% of nominal. Therefore, all results submitted by this report are related to mean measured test concentrations of the active substance.

The mean-measured concentration of the highest exposed test-solution (42.7 mg a.s./L) reflects the limited water-solubility under test conditions.
At the start of the study, the following parameters of the test water were determined: conductivity: 594 µS/cm, total hardness: 11° dH (= 196 mg/L $CaCO_3$) and alkalinity: 3° dH (= 53 mg/L $CaCO_3$ equivalent to 1 mL 0.1 N HCl). The measured pH of the test water as used for preparation of the test-solutions was 6.0. During the test, dissolved oxygen concentrations ranged from 96 to 102% oxygen saturation, the pH values ranged from 6.3 to 6.7 and the water temperature ranged from 20.5°C to 20.6°C.
Since the highest tested concentration of 100 mg a.s./L caused effects distinctly below 50% immobilisation, statistical evaluations are not applicable.

Toxicity to *Daphnia magna*:

Test Concentration		Exposed	Immobilised Daphnids			
Nominal	Mean Measured	Daphnids	24 h		48 h	
[mg a.s./L]	[mg a.s./L]	(= 100%)	n	% ± SD	n	% ± SD
Control	<0.636	30	0	0	0	0
Solvent Control	<0.636	30	0	0	0	0
6.25	4.70	30	0	0	0	0
12.5	8.77	30	0	0	0	0
25	20.3	30	0	0	0	0
50	38.8	30	0	0	2	7 ± 10
100	42.7	30	0	0	7	23 ± 23

B. Observations

During the first 24 hours of exposure, no immobilisation or other behavioural impacts occurred. At the 100 mg a.s./L concentration, precipitation was noted on the bottom of the container.

CONCLUSION

As the maximum exposure concentration caused only 23% immobility after 48 hours of static exposure, an EC_{50} calculation was not possible. Therefore it can be concluded, that the 48 h EC_{50} for *Daphnia* exposed to spirotetramat is greater than 42.7 mg a.s./L.

Effects of metabolites to *Daphnia magna*

Report:	**KIIA 8.3.1.1/02, [redacted]; 2005**
Title:	Acute Toxicity of BYI 08330-enol to the Waterflea *Daphnia magna* in a Static Laboratory Test System.
	Date: 2005-07-14
Organisation:	[redacted], Germany
Report No.:	EBFNM012; M-254487-01-2
Publication:	Unpublished
Dates of experimental work:	December 20, 2004 - January 14, 2005
Guidelines:	OECD - 202,April 4, 1984 and corresponding revised draft proposal, dated February 01, 2004; EEC Directive 92/69/EWG, part C.2; U.S.

	EPA Pesticide Assessment Guidelines, Subdivision E, § 72 2, OPPTS Guideline 850.1010 public draft 1996 (modified); JMAFF 12 Nousan No. 8147 (2000).
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to verify the absence of treatment-related effects on mobility of *Daphnia magna* over 48 hours in a static exposure, when exposed to a limit concentration of 100 mg pure metabolite/L. *Daphnia magna* (1st instars < 24 h old, 6 x 5 animals per concentration) were exposed in a static test system for 48 hours to a nominal limit concentration of 100 mg pure metabolite/L (corresponding to a mean-measured concentration of 111.5 mg p.m./L) without feeding. As measurement show, the physical / chemical properties of the test water (pH, temperature, oxygen-saturation, hardness) corresponded to the aspired values. Recoveries of BYI 08330-enol were measured at start and end of the 48 hours exposure period and all results are given as nominal concentrations.
The EC_{50} for immobility after 48 hours of static exposure is greater than 100 mg p.m./L.

MATERIAL AND METHODS

A Materials

1. Test material

Description	**Beige powder**
Lot/batch No.	**Batch no.: 692-101-09-0005** **Tox no.: TOX 06850-00**
Purity	**99.1%**
Stability of test compound	**Approved until 2005-03-17**
Water solubility	**1.751 mg/L (pH 7)**

2. Vehicle and/or positive control — **Test and breeding water was prepared as "ELENDT M7 medium".**

3. Test animals

Species	***Daphnia magna***
Age	**1st instars (< 24 h old)**
Source	**Strain from the [redacted] Germany, from laboratory stock breeding**
Acclimation period	**This breeding strain has been maintained in the test facility successfully for more than fifteen years in weekly renewed aqueous media. The first instars used in the test are obtained by repeated careful screening of an in-house breeding-culture of defined age less than 24h before test initiation. They descend from at least the fourth (or later) brood of parent daphnids (21 days ± 12 hours old). No males, ephippia or**

	dead animals were present in the cultures. The cultures showed no delay in first brood.
Environmental conditions	
Temperature	**20 ± 1°C**
Photoperiod	**16:8 hours, approx. 1500 Lux**

B Study design and methods

1. In life dates **December 20, 2004 - December 23, 2004**

2. Experimental treatments

***Daphnia magna* are kept in 2 L glass-containers (50 to 100 daphnids per container) in a climate controlled environment at 20 ± 1°C and a 16: 8 hour light-dark cycle. They were fed three times per week with the single cell green algae *Desmodesmus subspicatus*, supplemented by a commercial fish food (trade name: TetraMin®) in aqueous suspension.**

Based on the results of a range finding test, the actual study was intended as limit test according to the recommendations of the underlying guidelines, including a single exposure concentration of 100 mg pure metabolite L to be tested simultaneously with an untreated dilution water (blank) control.

To enhance the limited water-solubility of BYI 08330-enol, the pH of the test water was adjusted to pH 7 before use for dilution procedure. Preparation of test solutions occurred immediately before the start of exposure.

The test vessels consisted of 100 mL glass beakers, filled with 50 mL of the test solution. Six vessels (replicates), each provided with five daphnids (= 10 mL test solution per daphnid), were used per treatment group and controls (=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. 1500 lux. The waterfleas were exposed to BYI 08330-enol over a 48 hours period under static conditions.

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

3. Observations

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 possible signs of sublethal effects had to be recorded.

Prior to each preparation of test concentrations, conductivity, total hardness and alkalinity of the dilution media (ELENDT M7) were measured. The dissolved oxygen and pH values were measured in the freshly prepared test solutions of each treatment level and control and in the pooled replicates of the aged media at test termination (day 2). Light intensity was measured at start of the study with a

photometer. Environmental temperature was recorded continuously with a thermohygrometer. 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 the study.
For analytical verification of the test item concentrations, duplicate samples of the freshly prepared test media have been taken on day 0 from all test levels and the controls prior to introducing the test animals. For additional stability measurements the aged replicates of each remaining concentration level were pooled after 48 hours of exposure and samples were then taken from this pooled media at study termination. The limit of quantification (LOQ) for the method used (in water by HPLC-UV) was 5 µg/L. The method was validated within the current study.

RESULTS AND DISCUSSION

A. Findings

No immobilisation of untreated control animals occurred. Sensitivity of the used daphnid breeding-strain is located within the required range as verified by periodically performed reference substance testing. Thus, the study conditions and breeding quality met the required quality criteria.
The actual concentrations of BYI 08330-enol as measured for the freshly prepared test solutions (110 mg p.m./L) as well as for the corresponding aged media at the end of exposure (113 mg p.m./L) exceeded slightly the proposed nominal concentration.
Due to the clear results of the study (no treatment related effects evident up to a nominal test concentration of 100 mg pure metabolite/L), statistical evaluations were not performed.

Toxicity to *Daphnia magna*:

Test Concentration [mg p.m./L]		Exposed Daphnids (= 100%)	Immobilised Daphnids	
Nominally	Mean Measured		24 h	48 h
Control	< LOQ	30	0	0
100	111.5	30	0	0

p.m. pure metabolite

B. Observations

Observations on sublethal effects revealed no abnormal behaviour of the exposed daphnids over the whole exposure period of 48 hours and no immobility or other effects on behaviour occurred at or below the tested limit concentration of 100 mg p.m./L (nominally).

CONCLUSION

The EC_{50} for immobility of *Daphnia* exposed to BYI 08330-enol after 48 hours of static exposure is greater than 100 mg p.m./L.

Title:	4-Methoxycyclohexanone, Acute *Daphnia* toxicity Date: 2006-07-11
Organisation:	Bayer Industry Services, Leverkusen, Germany
Report No.:	2006/0032/01; M-274275-01-1
Publication:	Unpublished
Dates of experimental work:	June 13, 2006 – July 10, 2006
Guidelines:	EEC Directive 92/69/EEC, part C.2, in most parts equivalent to the OECD No. 202
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the influence of the test item on mobility of *Daphnia magna* over 48 hours in a static exposure, expressed as EC_{50} for immobilisation. In a limit test, *Daphnia magna* (neonates, 10 x 2 animals per concentration) were exposed in a static test system for 48 hours to nominal concentrations of 0 and 100 mg p.m./L without feeding (corresponding to measured concentrations of < 0.05 and 90.4 - 103.3 mg p.m./L, respectively). As measurements show, the physical/chemical properties of the test water (pH, temperature, oxygen saturation) corresponded to the aspired values. Due to analytical findings all results are given as nominal test concentrations of the test substance.

No immobility occurred after 48 hours of static exposure at the tested concentration, and the EC_{50} for 4-Methoxycyclohexanone is thus determined to be > 100 mg p.m./L.

MATERIAL AND METHODS

A Materials

1. Test material	4-Methoxycyclohexanone
Description	Not stated
Lot/batch No.	Batch no.: SAV5506-001
Purity	97.9%
Stability of test compound	Verified by chemical analysis (GC) at 0, 24 and 96 hours
2. Vehicle and/or positive control	No vehicle and/or positive control
3. Test animals	
Species	*Daphnia magna* STRAUS
Age	Neonates
Source	Strain of Bundesgesundheitsamt in Berlin, Germany, from laboratory stock breeding
Acclimation period	The breeding strain has been maintained in the test facility successfully for more than 15 years in weekly renewed aqueous media (M4).The neonates used in the test are obtained by filtration of the in-house breeding-culture of defined age prior to test initiation.
Environmental conditions	
Temperature	18 – 22°C (mean +/- 1°C)

Photoperiod	16 hours light / 8 hours dark, < 1000 lux

B Study design and methods

1. In life dates June 19, 2006 - July 10, 2006
2. Experimental treatments

Daphnia magna were kept at 20 ± 1°C and a 16 : 8 hour light-dark cycle. They were fed with unicellular green algae (*Desmodesmus subspicatus*) *ad libitum*.

The limit test concentration was 100 mg p.m./L (nominal). To achieve this concentration, the respective amount of the test item was added to 1 L of dilution water (reconstituted water, M4) and treated with an ultra turrax for 60 sec at 8000 rpm. Untreated dilution water (control) was run in parallel. The test medium was not renewed during the test.

The test vessels were 50 mL glass beakers, filled with 20 mL of the test solution. Two vessels (replicates), each provided with 10 daphnids (= 2 mL test solution per daphnid), were used per treatment group. The beakers were exposed at 18 - 22°C (± 1°C). They were illuminated at a 16:8 hours light dark cycle at a light intensity of ~1000 lux. The waterfleas were exposed to 4-Methoxycyclohexanone over a 48 hours period under static conditions.

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

3. Observations

After 24 and 48 hours, behaviour of the water fleas was visually evaluated by observing swimming ability and immobilisation rate.

Dissolved oxygen, pH values and temperature of the test media were measured at the end of the study in both control and treatment.

For analytical verification of the test item concentrations, aliquot samples of the test media were taken after 0 and 48 hours from both control and treatment.

RESULTS AND DISCUSSION

A. Findings

No immobilisation of untreated control and test animals occurred. The measured concentrations of 4-Methoxycyclohexanone in the test solutions at test initiation ranged between 90.4 and 103.3% of the corresponding nominal concentrations. Therefore, all results are related to nominal test concentrations of the test substance.

Total hardness of the dilution water, measured at test start, was 15.3° dH (= 273.1 mg/L $CaCO_3$). At the end of the test, dissolved oxygen concentrations were 98% oxygen saturation, the pH values ranged from 7.8 to 9.2 and the water temperature ranged from 21.4°C to 21.5°C.

Since the test concentration of 100 mg p.m./L caused no effects on immobilisation, thus distinctly below 50%, statistical evaluations are not applicable.

Toxicity to *Daphnia magna*:

Test Concentration [mg p.m./L]		Exposed Daphnids (= 100%)	Immobilised Daphnids	
Nominally	Measured		24 h	48 h

Control	<0.05	20	0	0
100	90.4 – 103.3 (0 h – 48 h)	20	0	0

B. Observations

During the exposure, no immobilisation or other behavioural impacts occurred.

CONCLUSION

As the exposure concentration of 100 mg p.m./L caused no immobility after 48 hours of static exposure, an EC_{50} calculation was not possible. Therefore it can be concluded that the 48 h EC_{50} for *Daphnia* exposed to 4-Methoxycyclohexanone is > 100 mg p.m./L.

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

The acute toxicity of spirotetramat is investigated for *Daphnia magna*. As spirotetramat is not intended to be used directly on surface water, additional data for aquatic insects are not deemed necessary.

IIA 8.3.1.3 Acute toxicity for representative species of aquatic crustaceans

The acute toxicity of spirotetramat is investigated for *Daphnia magna*. As spirotetramat is not intended to be used directly on surface water, additional data for aquatic crustaceans (on a species not related to *Daphnia*) are not considered necessary.

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

The acute toxicity of spirotetramat is investigated for *Daphnia magna*. As spirotetramat is not intended to be used directly on surface water, additional data for aquatic gastropod molluscs are not considered necessary.

IIA 8.3.2 Chronic toxicity to aquatic invertebrates

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

Report: **KIIA 8.3.2.1/01, [redacted]; 2005**
Title: Influence of BYI 08330 (tech.) on Development and Reproductive Output of the Waterflea *Daphnia magna* in a Static Renewal Laboratory Test System.
Date: 2005-05-13
Organisation: [redacted], Germany
Report No.: EBFN0245; M-251843-01-2
Publication: Unpublished
Dates of experimental work: November 16, 2004 – December 13, 2004

Guidelines:	OECD-211, dated September 21, 1998: *Daphnia magna* Reproduction Test, EEC Directive 92/69/EWG, part C.20, U.S. EPA-Pesticide Assessment Guidelines, Subdivision E, §72-4, dated October, 1982: aquatic invertebrate life-cycle studies, U.S. EPA- OPPTS Guideline 850.1300, dated April 1996: Daphnid chronic toxicity test- public Draft, JMAFF 12 Nousan No. 8147 (2000): Determination of the Inhibition of the Mobility of Daphnia (2-7-2).
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the influence of the test item on development, reproductive capacity and behaviour of *Daphnia magna* over 21 days under static-renewal exposure, expressed as chronic NOEC for parental health and reproductive output.
Daphnia magna (1st instars < 24 h old, 10 x 1 animal per concentration) were exposed in a static-renewal test system (renewal of test solution 3 times per week) for 21 days to nominal concentrations of 12.5, 5.0, 2.0, 0.8, 0.32, 0.13 and 0.05 mg a.s./L (corresponding to calculated time-weighted-mean concentrations of 10.9, 4.41, 1.84, 0.796, 0.322, 0.129 and 0.0553 mg a.s./L). For water quality monitoring, temperatures, pH values and O_2 concentrations, conductivity, hardness and alkalinity of the test solutions, were regularly controlled throughout the study as recommended by the underlying guidelines. As measurements show, the physical/chemical properties corresponded to the required values.
The endpoints recorded as database for NOEC/LOEC/MATC calculation were:
the number of living offspring per surviving parental animal, the parental age at first offspring emergence as well as the rate of parental survivors and their body-length at the end of the study.
Additional assessments were made for adverse effects on neonate's behaviour and parental body-mass at the end of the study.
The overall chronic NOEC for 21 days of static renewal exposure of spirotetramat to *Daphnia magna* expressed as nominal test concentration is 2.0 mg a.s./L, based on parental immobilisation.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014 (mix-batch)** **Tox no.: TOX 06689-01**
Purity	**97.1%**
Stability of test compound	**Expiration date: 2005-05-05**
2. Vehicle and/or positive control	**Test and breeding water was prepared as "ELENDT M7 medium".** **Acetone was used as solvent for the test item.**

3. Test animals

Species	***Daphnia magna***
Age	**1st instars (< 24 h old)**
Source	**Strain from the Bundesgesundheitsamt in Berlin, Germany, from laboratory stock breeding** **This breeding strain has been maintained in the test facility successfully for more than fifteen years in weekly renewed aqueous media.**
Acclimation period	**The first instars used in the test are obtained by repeated careful screening of an in-house breeding culture of defined age less than 24h before test initiation. They descend from at least the fourth (or later) brood of coeval parent daphnids (20 days ± 12 hours old). No males, ephippia or dead animals were present in the cultures. The cultures showed no delay in first brood.**
Environmental conditions	
Temperature	**20 ± 1°C**
Photoperiod	**16:8 hours, approx. 1500 Lux**

B Study design and methods

1. In life dates November 16, 2004 – December 13, 2004

2. Experimental treatments

The parental water fleas were exposed to BYI 08330 (tech.) over a 21 days period under static renewal conditions. 7 concentrations (12.5, 5.0, 2.0, 0.8, 0.32, 0.13 and 0.05 mg a.s./L), a water control (test medium without test item added) and an additional solvent control were tested. Due to the limited water-solubility of the test item, a solvent amount of 0.2 mL acetone/L test-solution was admixed to all exposure media except untreated control.

The test vessels were 250 mL glass beakers, filled with 100 mL of the test solution. Ten vessels (replicates) were used for each concentration and controls. Test solutions were renewed in 48 hours (during the week) respectively 72 hours (weekend) intervals. The test solutions were not artificially aerated during the test. All test vessels were placed in a climate controlled environment (isolated chamber) at temperatures of 20 ± 1 °C. They were illuminated by cool white fluorescent bulbs in a 16:8 hours light dark cycle at a light intensity of max. 1500 lux.

Over the whole exposure period, the water fleas were fed daily, except the first weekend (study days 3 & 4). The food consisted of living single cell green algae (*Desmodesmus subspicatus*).

For analytical verification of the test item concentrations, samples were taken on day 0, 9 and day 19 from all test levels and controls prior to introducing the food and test animals.

The aged replicates were pooled on days 2, 12 and 21 for analysis, giving an indication of stability in the test system over 2 or 3 days. The limit of quantification (LOQ) for the method used was 5 µg/L. The method was validated within the current study.

3. Observations

Visual evaluations were performed daily, with exception of Saturday and Sunday of the first week (days 3 and 4). Observations on parent mobility and neonate survival were executed (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 possible signs on sublethal effects were recorded. Starting with day 6, the freshly emerged neonates were counted and removed daily. The onset of maturity was recorded separately for each parental female.

At study termination, the body length of all surviving parent animals was determined by measurement under a scaled binocular. Complementarily, parental body mass was determined as dry weight after individually deep frozen samples have been lyophilised for at least 24 hours.

Prior to each preparation of test concentrations, conductivity, total hardness and alkalinity of the dilution media (ELENDT M7) were measured. Water temperatures, content of dissolved oxygen, pH values, conductivity, total hardness and alkalinity were measured in the freshly prepared test solutions of each

treatment level and controls (electronic measuring equipment) and repeatedly in the aged media at the end of each two or three days lasting exposure interval (pooled replicates).
Before evaluation of the daily observation data, the parental survival rates (mobile animals) were initially arc sine transformed. The reproductive output data and the transformed mobility data were analysed on variance homogeneity (Bartlett's Test) and normal distribution (Kolmogorroff-Smirnov Test) on a 5% level of significance using the treatment levels and pooled controls as co-variates.
If homogeneity / equality of variances was confirmed, subsequent analyses were conducted using parametric techniques on a 5% level of significance ($p < 0.05$).
Parametric procedures involved subjecting reproduction data to a standard one way analysis of variance (ANOVA). If significant differences among the means were indicated, multiple comparison procedures (e.g. Dunnett's multiple t-test procedure or adequate step down trendtests (e.g. Williams multiple sequential t-test procedure) were performed on a 5% level of significance ($p < 0.05$ / one-sided [smaller] probability), to indicate which treatment groups differed significantly from the control.
For non parametric procedures, the Mann-Whitney-Wilcoxon U-test for independent samples was applicable, which allows a pairwise comparison of the medians from unpaired datasets of different sizes. Alternatively, corresponding multiple comparison procedure with Bonferroni-Correction was applicable.
All described statistical procedures are carried out by using the ToxRat Professional® Software, Vers.2.09 of the ToxRat Solutions GmbH, Germany.

RESULTS AND DISCUSSION

A. Findings

No immobilisation of untreated control animals occurred. Sensitivity of the daphnid breeding-strain used is located within the required range as verified by periodically performed acute reference substance testing. The reproductive output as recorded for both control groups fulfilled the required minimum value of > 60 neonates per female during 21 days. The data for all study endpoints revealed homegeneity between both control groups. Thus, the data from both control groups were pooled and all data from treatment groups were related to the pooled control data.
The study conditions and breeding quality met the required quality criteria.
Based on 3 series of weekly measurements, the actual concentrations of BYI 08330 in freshly prepared and aged media were determined from all concentration levels. From the measured results, the time weighted mean concentrations over 21 days of exposure were calculated. The corresponding values ranged between 87.2 and 111% of the proposed nominal concentrations. Since the time weighted mean concentrations matched nominal values closely, all reported results are based on nominal values.
All measured values for control groups were found to be below the lowest analytical-standard concentration during analysis of the test samples (0.0136 mg/L).

Effects of spirotetramat on reproduction, growth and immobilisation of *Daphnia magna* in a static-renewal 21-days chronic toxicity test, based on nominal concentrations is shown in the following table:

Biological Results (mean-values by study group)

Treatment [mg a.s./L] (nominal)	Parental endpoints			Reproductive endpoints		
	body length [mm]	body mass [mg]	survival [%]	cumulative offspring per parent animal	parental age at first offspring emergence [days]	neonates behaviour [% unaffected neonates]
Water Contr.	4.50	1.06	100	107.3	9.1	100
Solv. Contr.	4.42	0.98	100	115.2	9.2	100
Pooled Contr.	4.46	1.02	100	116.3	9.2	100
0.05	4.55	1.09	100	127.3	9.6	100
0.13	4.56	0.89	100	127.4	8.5	100
0.32	4.55	0.90	100	113.0	9.0	100
0.80	4.58	1.00	100	118.7	9.4	100
2.00	4.60	1.04	100	122.4	9.5	100
5.00	4.64	1.04	90	115.2	9.6	100
12.50	4.21	0.78	80	93.5	9.5	100

The biological endpoints, as recommended by the underlying Guidelines, revealed the following threshold concentrations:

for the cumulative offspring per surviving parent animals:

no observed effect concentration (NOEC)	5.0 mg a.s./L
lowest observed effect concentration (LOEC)	12.5 mg a.s./L

for immobilisation of the parent animals:

no observed effect concentration (NOEC)	2.0 mg a.s./L
lowest observed effect concentration (LOEC)	5.0 mg a.s./L

for the parental litsage at first offspring emergence:

no observed effect concentration (NOEC)	≥ 12.5 mg a.s./L
lowest observed effect concentration (LOEC)	> 12.5 mg a.s./L

for final body length of surviving parental animals:

no observed effect concentration (NOEC)	5.0 mg a.s./L
lowest observed effect concentration (LOEC)	12.5 mg a.s./L

for final body mass of surviving parental animals:

no observed effect concentration (NOEC)	5.0 mg a.s./L
lowest observed effect concentration (LOEC)	12.5 mg a.s./L

CONCLUSION

The overall chronic NOEC for 21 days of static renewal exposure of spirotetramat to *Daphnia magna* expressed as nominal test concentration is 2.0 mg a.s./L. This NOEC based on parental immobilisation as caused by treatment levels of 5.0 mg a.s./L (LOEC) and 12.5 mg a.s./L. Therefore the "Maximum Acceptable Toxicant Concentration" (MATC), calculated as geometric mean between NOEC and LOEC, is 3.2 mg a.s./L (nominal).

IIA 8.3.2.2 Chronic toxicity for representative species of aquatic insects

For studies on *Chironomus riparius* see point 8.5. Further studies are not triggered as spirotetramat is not intended to be applied directly on surface water.

IIA 8.3.2.3 Chronic toxicity for repr. species of aquatic gastropod molluscs

Studies on chronic toxicity for aquatic gastropods molluscs are not required as spirotetramat is not intended to be applied directly on surface water.

IIA 8.3.3 Aquatic field testing*

The performed studies with active substance allow a complete assessment of effects on aquatic organisms. Thus, no further studies are deemed necessary.

IIA 8.4 Effects on algal growth and growth rate (2 species)

Report:	**KIIA 8.4/01, [redacted]; 2004**
Title:	*Pseudokirchneriella subcapitata* Growth Inhibition Test with BYI 08330 (tech.)
	Date: 2004-11-29
Organisation:	[redacted], Germany
Report No.:	DOM 23092; M-128874-01-2
Publication:	Unpublished
Dates of experimental work:	April 30, 2004 - June 25, 2004
Guidelines:	Draft Proposal for Updating OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (Feb. 18, 2004) EU Council Directive 91/414/EEC (1991) JMAFF guideline (12 Nousan No 8147 (2000)) OPPTS Guideline 850.5400 Draft (July 1996) U.S. EPA Pesticide Assessment Guidelines, Subdivision J, § 122-2, 123-2
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* (freshwater microalgae, formerly known as *Selenastrum capricornutum*) expressed as NOEC, LOEC and ECx for growth rate of algal biomass (cells per volume). Per US EPA requirements, the same endpoints are also calculated for yield based on

*** No EC data requirement (the OECD point concerned is not covered by or part of an EC point according to Council Directive 91/414/EEC. Hence, data/documents do not need to be submitted.**

algal biomass. *Pseudokirchneriella subcapitata* were exposed in a multigeneration test for 3 days under static exposure conditions to the nominal concentrations of 0.31, 1.0, 3.1, 10, 31 and 100 mg a.s./L in comparison to controls, corresponding to mean-measured concentrations of <0.0317, 0.0679, 0.242, 0.752, 3.18, 12.1 and 47.9 mg a.s./L.
The pH values ranged from 7.8 to 8.3 in the controls, the incubation temperature and the continuous illumination corresponded to the aspired values. Due to analytical measurements in all treatment groups on day 0 and day 3 of the exposure period all results are given as mean measured test concentrations of the active substance.
The (0 - 72h)-E_rC_{50} for BYI 08330 is 8.15 mg a.s./L (95% Cl: 7.56 - 8.81) and the (0 - 72h)-NOE_rC is 1.46 mg a.s./L. The 72h E_yC_{50} for BYI 08330 was 5.6 mg a.s./L.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014 (mix-batch)** **Tox no.: 06344-01**
Purity	**97.4%**
Stability of test compound	**Sample released until 2004-06-04**
2. Vehicle and/or positive control	**The medium was freshly prepared according to the mentioned test.** **Acetone was used as solvent for the test item.**
3. Test animals	
Species	***Pseudokirchneriella subcapitata* (freshwater microalgae, formerly known as *Selenastrum capricornutum*), strain SAG 61.81**
Age	**Exponentially growing inoculum**
Source	**Collection of Algal Cultures, Inst. for Plant Physiology, University of Göttingen, Göttingen, Germany. Transferred to the laboratory on July 15, 2002 and kept since then.**
Acclimation period	**An inoculum pre-culture was prepared 2-4 days before the start of the test and cultivated under the same conditions as in the main test.**
Environmental conditions	
Temperature	**23 ± 2°C**
Photoperiod	**Continuous illumination, 8000 lux (± 15%)**

B Study design and methods

1. In life dates **April 26, 2004 - April 30, 2004**

2. Experimental treatments

Once per week 200 µL of a 7-9 days old algae stock culture was transferred into a 250 mL cotton plugged Erlenmeyer flask containing 50 mL of nutrient medium. Stock cultures of algae were kept at 23 ± 2°C with 16 h light/day. All operations

were conducted under sterile conditions to handle an axenic algae culture. To ensure that the algae used as inoculum were exponentially growing, an inoculum pre-culture was prepared 2-4 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 test vessels consisted of 300 mL Erlenmeyer flasks, filled with 150 mL nutrient medium and inoculated algae cells. They were placed on a tablet rotating 100 rpm to prevent sedimentation of the cells without additional aeration. The incubation temperature was 22.5 to 23.1°C over the whole period of testing at a continuous illumination.

The stock solution contained 10.252 g BYI 08330 (tech.) with 50 mL acetone, prepared immediately prior to the test. The stock solution was well agitated on a magnetic stirrer for 10 minutes and for 10 minutes in an ultrasonic bath before further use. An adequate amount of the stock solution was transferred to a dilution series with acetone to obtain the concentration levels used in the study.

The actual study included 3 replicate vessels per test level (6 replicate vessels per control). The range of the test concentrations was selected based on pre experiments: 0, 0.3, 1.0, 3.1, 10, 31, and 100 mg a.s./L (nominal). The test duration accounted to 3 days.

3. Observations

Morphological examinations of cells by a microscope were made over the exposure period on each study day.

For biomass quantification cell numbers per volume (as a surrogate for biomass per volume) were estimated photometrically on day 1, day 2, and day 3 of the exposure period (without replacing after measurement). The extinctions were determined at a wave length of 578 nm using a single-beam-photometer.

The temperature was determined by a continuous measurement in one additional incubated glass vessel filled with the same amount of de-ionised water as in the test vessels. The pH was measured at least at the beginning and at the end of the exposure period in all test levels and the controls.

For analytical verification samples were analysed for the actual concentration of BYI 08330 present in the test medium at all treatment levels and the controls on day 0 and day 3. At exposure termination, therefore the contents of all replicate vessels were combined. The limit of quantitation (LOQ) for the method used (in water by HPLC-UV) was 0.05 µg/L. The method was validated within the current study.

EC_X values and confidence intervals were calculated for the stated exposure period, using a commercial program (ToxRat Professional©). The LOEC determinations from the appropriate parameter (inhibition) were done using the ANOVA procedure (p = <0.05, one sided) and properly selected multiple t-tests.

A. Findings

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

The analytical findings of BYI 08330 in the treatment levels found on day 0 were 84 to 94% of nominal (average 90.7%). On day 3 analytical findings of 22 to 48% of nominal (average 31.5%) were found. The low analytical findings on day 3 are explained by the hydrolytic half-life of BYI 08330 under alkaline conditions of testing. Therefore all reported results are based on the geometric mean values of the test item during exposure.

The pH values ranged from 7.8 to 8.3 in the controls and the incubation temperature ranged from 22.5°C to 23.1°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7414 lux.

The static 72 hour algae growth inhibition test resulted in the following tabulated effects:

Geometric mean [mg a.s./L]	Cell Number after 72 h (means) per mL	(0-72h) Average Specific Growth Rate [$days^{-1}$]	Inhibition of Average Specific Growth Rate [%]	Doubling time of algae cells [days]
Pooled controls	468,000	1.279		0.542
0.141	719,000	1.424	- 11.3	0.487
0.471	570,000	1.347	- 5.3	0.515
1.46	508,000	1.307	- 2.2	0.530
5.38	254,000	1.047	18.1	0.662
18.5	5,000	0.291	122.7	-
63.2	3,000	- 0.393	130.7	-

test initiation with 10,000 cells/mL

Calculation of Yield (in fulfillment of US EPA requirements)

Geometric mean Concentration (mg a.s./L)	Cell Number after 0 h (means) per mL	Cell Number after 72 h (means) per mL	Yield	Inhibition of yield in %
Pooled controls	10,000	468,000	458 000	--
0.141	10,000	719,000	709 000	-54.8
0.471	10,000	570,000	560 000	-22.3
1.46	10,000	508,000	498 000	-8.7
5.38	10,000	254,000	245 000*	46.7
18.5	10,000	5,000	-6 000*	101.1
63.2	10,000	3,000	-7 000*	101.5

- % inhibition: increase in growth relative to the control

*significantly (α=0.05, one-sided smaller) reduced, based on Dunnett's Multiple t-test

B. Observations

In the highest test level of 63.2 mg a.s./L (nominal test concentration: 100 mg a.s./L) the test item was not dissolved completely, which can be explained by the limited solubility of the test item.

CONCLUSION

The (0 - 72 h)-E_rC_{50} for spirotetramat is 8.15 mg a.s./L (95% CI: 7.56 - 8.81) and the (0 - 72 h)-NOE_rC is 1.46 mg a.s./L (based on the geometric mean of measured concentrations). The E_yC_{50} at 72h for BYI 08330 was 5.6 mg a.s./L.

Report:	**KIIA 8.4/02, [redacted], and [redacted]; 2005**
Title:	Toxicity of BYI 08330 to the Freshwater Diatom *Navicula pelliculosa*
	Date: 2005-06-15
Organisation:	Bayer CropScience, Ecotoxicology, South Metcalf, Stilwell, Kansas
Report No.:	EBFNX008; M-252794-01-1
Publication:	Unpublished
Dates of experimental work:	February 21, 2005 – February 25, 2005
Guidelines:	FIFRA Guideline 123-2 (1982) OPPTS Guideline 850.5400 (1996 draft) OECD Guideline 201 (1984, 2004 draft)
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

A static 96-hour algal growth test was conducted to determine the growth effects of spirotetramat to the freshwater diatom *Navicula pelliculosa*. *Navicula pelliculosa* was exposed under static (shaken cultures) conditions for 96-hours to nominal concentrations of 0.20, 0.51, 1.3, 3.2, 8.0 and 20.0 mg a.s./L, corresponding to initial measured concentrations of <0.02 (control), <0.02 (solvent control), 0.22, 0.47, 1.25, 3.20, 8.23 and 20.4 mg a.s./L.

The physico/chemical parameters corresponded to the aspired values. Due to analytical measurements all results based on initial measured concentrations.

The 72 hour EC_{50} value for growth rate (E_rC_{50}) was calculated to be 12.17 mg a.s./L. The 72 hour NOEC is 3.20 mg a.s./L.

MATERIAL AND METHODS

A Materials

1. Test material	**BYI 08330 (tech.); synonym: AE 1302943**
Description	**Solid white powder**
Lot/batch No.	**Batch no.: 08045/0014**
Purity	**97.1%**
Stability of test compound	**Expiration date: 2005-05-05 when stored at approx. 20°C**
Water solubility	**300 mg/L at 25.0 °C and pH 7**
2. Vehicle and/or positive control	**Acetone was used as solvent for the test item (0.5 mL/L).**
3. Test animals	
Species	***Navicula pelliculosa* (freshwater diatom)**
Age	**Exponentially growing inoculum**
Source	**University of Texas (UTEX), Austin, Texas, US**
Acclimation period	**Inoculum source: in-house 3 day old batch culture held under test conditions**
Environmental conditions	
Temperature	**24 ± 2.0°C**
Photoperiod	**24 hour light; 4026 to 4693 lux**

B Study design and methods

1. In life dates February 21, 2005 – February 25, 2005

2. Experimental treatments

An initial cell density of 10,000 cells/mL was used. The test vessels were sterile 250-mL Erlenmeyer flasks covered by inverted glass beakers, filled with 50 mL of the test solution. Test media was nutrient media 1 x AAP with 3 x silicates, prepared with soft process water with an initial pH of 7.5 ± 0.1.

The stock solution contained 4.1152 g BYI 08330 (tech.) diluted in acetone, resulting in a stock solution volume of 100 mL. The stock solution was mixed by sonication for 25 minutes. An adequate amount of the stock solution was transferred to a dilution series with acetone to obtain the concentration levels used in the study. The solvent load was 0.5 mL acetone/L.

The actual study included 3 replicate vessels per test level. Based on a range-finding test, the nominal test concentrations were Control, Solvent Control, 0.20, 0.51, 1.3, 3.2, 8.0 and 20 mg a.s./L. The test vessels were not aerated. The test duration was 4 days.

3. Observations

For each study day, density was determined in the three replicates at each test concentration using a model Z1 Beckman Coulter® particle counter. This was accomplished by first counting a culture of algae cells using a hemocytometer. The limit of detection for the hemocytometer was 1×10^4 alga cells/mL. After 72 and 96 hour the growth rate (NOEC and EC_{50}) was determined, additionally after 96 hour the standing crop and cumulative biomass. Cellular observations were conducted daily by visual inspection via light microscope.

The temperature was determined by a continuous measurement in one additional incubated 250 mL flask of water centrally located in the environmental chamber. The interval of pH and conductivity measurements was day 0 and day 4.

For analytical verification samples were analysed for the actual concentration of BYI 08330 present in the test medium at all treatment levels and the controls on day 0 (from batch preparation) and day 4 (composited samples from each level). The limit of quantitation (LOQ) for the method used (in water by HPLC-UV) was 0.02 mg/L. The method was validated within the current study.

Raw or transformed data from treatment groups were compared to controls for normality and homogeneity of variance using the Shapiro-Wilks test and Levene's test of equal variance, respectively. If normality and homogeneity of variance were demonstrated for the raw or transformed values, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test. The 72 or 96-hour EC_{50}, and the respective 95% confidence intervals, will be calculated with help of regression analysis for cell density, cumulative biomass, and growth rate. The commercial computer program SAS 1999 was used for statistical evaluation.

RESULTS AND DISCUSSION

A. Findings

Validity criteria were met as the cell culture density in the control cultures increased exponentially by a factor of 150 over the 0-72-hour test period (required: 16), the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures was 7% (required ≤ 35%) and the coefficient of variation of average specific growth rates during the 0-72 hour period between the replicate control cultures was 3% (required ≤ 7%).

The pH values ranged from 7.7 to 9.5, the temperature (recorded hourly) from 23.5 to 24.1°C and conductivity from 236 to 259 µmhos/cm.

Initial measured recoveries were within the range of 92 to 112% of the nominal concentrations while the day 4 measured concentration range was between 0 and 59% of the nominal test concentrations. The toxicity values were calculated based on the initial measured concentrations due to the low recovery rate of day 4 measurements.

Cell Density, Cumulative Biomass and Growth Rate During the Exposure of *Navicula pelliculosa* to BYI 08330 tech.

72-hour Endpoints		
Initial Measured Concentration [mg a.s./L]	Mean growth rate[a]	% Inhibition[b]
Control	0.069312	n.a.
Solvent Control	0.070174	n.a.
Pooled Controls	0.069743	n.a.
0.22	0.069227	0.7
0.47	0.069029	1.0
1.25	0.068980	1.1
3.20	0.067678**	3.0
8.23	0.052128*	25
20.4	0.012814*	82

96-hour Endpoints						
Initial Concentration [mg a.s./L]	Mean Density [cells/mL x 10^4]	%	Mean Cumulative Biomass	%	Mean Growth Rate[a]	%
Control	329.4	n.a.	8325.80	n.a.	0.060338	n.a.
Solvent	377.1	n.a.	9135.56	n.a.	0.061795	n.a.
Pooled	n.a.	n.a.	8730.68	n.a.	0.061067	n.a.
0.22	292.1*	23	7766.52	11	0.059123	3.2
0.47	305.4*	19	7874.24	10	0.059583	2.4
1.25	272.7*	28	7450.32*	15	0.058377**	4.4
3.20	287.7*	24	7291.20*	16	0.058949	3.5
8.23	169.6*	55	3340.72*	62	0.053384*	13
20.4	4.62*	99	117.24*	99	0.015824*	74

* Statistical significant difference from control (Dunnett's one-tailed test; $p \leq 0.05$).

**Statistical significant difference from control but not considered biologically significant

[a] Growth rate [1/h] is calculated from the cell density data.

[b] % Inhibition=100-((Treatment group parameter mean/control parameter mean)*100).

n.a. not applicable

Test substance	**Spirotetramat tech.**
Test object	***Navicula pelliculosa***
Exposure	**96 hour, static**
0-72-h E_rC_{50} - growth rate	**12.17 mg a.s./L (95% CI 11.74 to 13.64 mg a.s./L)**
96-h EC_{50} - cell density (standing crop)	**8.11 mg a.s./L (95% CI 7.14 to 9.22 mg a.s./L)**
96-h E_bC_{50} - cumulative biomass	**7.33 mg a.s./L (95% CI 6.65 to 8.09 mg a.s./L)**
96-h E_rC_{50} - growth rate	**15.10 mg a.s./L (95% CI 14.48 to 15.65 mg a.s./L)**
96-h LOEC - cell density (standing crop)	**0.22 mg a.s./L (initial measured)**
96-h LOEC - cumulative biomass	**1.25 mg a.s./L (initial measured)**
72-h LOEC - growth rate	**8.23 mg a.s./L (initial measured)**
96-h NOEC - cell density (standing crop)	**-**
96-h NOEC - cumulative biomass	**0.47 mg a.s./L (initial measured)**
72-h NOEC - growth rate	**3.20 mg a.s./L (initial measured)**

B. Observations

No physical abnormalities were observed in the controls or treatment groups during the study.

CONCLUSION

The 72-hour endpoints were calculated based on initial measured concentrations. The 72 hour EC_{50} value for growth rate (E_rC_{50}) is 12.17 mg a.s./L. The 72 hour NOEC is 3.20 mg a.s./L.

Report: **KIIA 8.4/03, [redacted], and [redacted]; 2006**
Title: Toxicity of BYI 08330 Technical to the Blue-Green Algae *Anabaena flos-aquae*.
Date: 2006-01-12

Organisation:	Bayer CropScience, Ecotoxicology, South Metcalf, Stilwell, Kansas
Report No.:	EBFNX007; M-264055-01-1
Publication:	Unpublished
Dates of experimental work:	May 23, 2005 – May 27, 2005
Guidelines:	FIFRA Guideline 123-2 (1982) OPPTS Guideline 850.5400 (1996 draft) OECD Guideline 201 (1984, 2004 draft)
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

A static 96-hour algal growth test was conducted to determine the growth effects of BYI 08330 technical to the blue-green algae, *Anabaena flos-aquae*. The algae was exposed under static (shaken cultures) conditions for 96-hours to nominal concentrations of control, solvent control, 0.51, 1.3, 3.2, 8.0 and 20.0 mg a.s./L.

The physico/chemical parameters corresponded to the aspired values. Due to analytical measurements all results based on initial measured concentrations.

The 72 hour EC_{50} value for growth rate (E_rC_{50}) was calculated to be 24.0 mg a.s./L. The 72 hour NOEC is 21.7 mg a.s./L.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**Solid white powder**
Lot/batch No.	**Batch no.: 08045/0014**
Purity	**97.99%**
Stability of test compound	**Expiration date: April 18, 2007 when stored at approx. 20°C**
Water solubility	**30.0 mg/L at 25.0 °C and pH 7**
2. Vehicle and/or positive control	**Acetone was used as solvent for the test item (0.5 mL/L).**
3. Test animals	
Species	***Anabaena flos-aquae* (blue-green algae)**
Age	**Exponentially growing inoculum**
Source	**University of Texas (UTEX), Austin, Texas, USA, received 2005-12-04**
Acclimation period	**Inoculum source: In-house 3 day old batch culture held under test conditions.**
Environmental conditions	
Temperature	**24 ± 2.0°C**
Photoperiod	**24 hours light; 2200 lux (± 10%)**

B Study design and methods

1. In life dates **May 23, 2005 – May 27, 2005**

2. Experimental treatments

An initial cell density of 10,000 cells/mL was used. The test vessels consisted of sterile 250-mL Erlenmeyer flasks covered by inverted glass beakers, filled with 100 mL of the test solution. Test media was nutrient media 1 x AAP, prepared with soft process water with an initial pH of 7.5 ± 0.1.

The stock solution contained 4.0823 g BYI 08330 (tech.) diluted in acetone, resulting in a stock solution volume of 100 mL. The stock solution was mixed by sonication for 30 minutes. An adequate amount of the stock solution was transferred to a dilution series with acetone to obtain the concentration levels used in the study. The solvent load was 0.5 mL acetone/L.

Based on the range find study results and the practical limit of solubility of the material, the definitive test concentrations were set to Control, Solvent Control, 0.51, 1.3, 3.2, 8.0, and 20 mg a.s./L. The actual study included 3 replicate vessels per test level. The test duration was 4 days.

3. Observations

For each study day, density was determined in the three test replicates at each test concentration using a light microscope and an Improved Neubauer hemocytometer. The limit of detection for the hemocytometer was 1 x 10^4 alga cells/mL. After 72 and 96 hour the growth rate (NOEC and EC_{50}) was determined, additionally after 96

hour the standing crop and cumulative biomass. Cellular observations were conducted daily by visual inspection via light microscope.
The temperature was determined by a continuous measurement in one additional incubated 250 mL flask of water centrally located in the environmental chamber.
The interval of pH and conductivity measurements was day 0 and day 4.
For analytical verification samples were analysed for the actual concentration of BYI 08330 present in the test medium at all treatment levels and the controls on day 0 (from batch preparation) and day 4 (composited samples from each level).
The limit of quantitation (LOQ) for the method used (in water by HPLC-UV) was 0.02 mg/L. The method was validated within the current study.
Raw or transformed data from treatment groups were compared to controls for normality and homogeneity of variance using the Shapiro-Wilks test and Levene's test of equal variance, respectively. If normality and homogeneity of variance were demonstrated for the raw or transformed values, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test. The 72- or 96-hour EC_{50}, and the respective 95% confidence intervals, will be calculated with help of regression analysis for cell density, cumulative biomass, and growth rate. The commercial computer program SAS, SAS Institute Inc., Cary, NC was used for statistical evaluation.

RESULTS AND DISCUSSION

A. Findings

The test conditions did not meet all validity criteria given by the mentioned guidelines. The cell culture density in the control cultures increased by a factor of approx. 44 over the 0-72-hour test period (required: 16), but the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures was 87% (required ≤ 35%) and the coefficient of variation of average specific growth rates during the 0-72-hour period between the replicate control cultures was 19% (required ≤ 7%). However, both criteria are often difficult to meet for this species due to chained growth which lends to increased variability.
The pH values ranged from 7.7 to 8.3, the temperature (recorded hourly) from 23.3 to 23.8°C and conductivity from 212 to 220 µmhos/cm.
Initial measured recoveries were within the range of 87 to 113% of the nominal concentrations, while the day 4 measured test concentrations ranged from 31 to 52%. The toxicity values were calculated based on the initial measured concentrations.

Cell Density, Cumulative Biomass and Growth Rate During the Exposure of *Anabaena flos-aquae* to BYI 08330 tech.

72-hour Endpoints		
Initial Measured Concentration [mg a.s./L]	Mean growth rate[a]	% Inhibition[b]
Control	0.045481	n.a.
Solvent Control	0.038312	n.a.
Pooled Controls	0.041896	n.a.
0.45	0.051117	-22
1.29	0.040437	3
3.32	0.050263	-20
9.05	0.046832	-12
21.7	0.030288**	28

96-hour Endpoints						
Initial Concentration [mg a.s./L]	Mean Density [cells/mL x 10^4]	%	Mean Cumulative Biomass	%	Mean Growth Rate[a]	%
Control	51.3	n.a.	1467.9	n.a.	0.040307	n.a.
Solvent	36.8	n.a.	1104.3	n.a.	0.036436	n.a.
Pooled	44.0	n.a.	1286.1	n.a.	0.038372	n.a.
0.45	39.6	10	1833.5	-43	0.037259	3
1.29	68.3	-55	1484.4	-15	0.043380	-13
3.32	75.7	-72	1935.8	-51	0.043291	-13
9.05	47.5	-8	1357.5	-6	0.039711	-3
21.7	24.72**	44	663.12**	48	0.032999**	14

* Statistical significant difference from control (Dunnett's one-tailed test; $p \leq 0.05$).
**Statistical significant difference from control but not considered biologically significant
[a] Growth rate [1/h] is calculated from the cell density data.
[b] % Inhibition=100-((Treatment group parameter mean/control parameter mean)*100).
n.a. not applicable

The exposure was conducted up to the maximal dose possible based on the practical limit of solubility for BYI 08330 (20 mg a.s./L) under these test conditions. While EC_{50} estimates were possible, the limited sensitivity of *Anabaena flos-aquae* to the test material resulted in a derived no observed effects concentration (NOEC) equal to that of the highest test concentration for all measured endpoints. However, this data provides adequate information to assess relative toxicity, and thus risk.

Test substance	**BYI 08330**
Test object	***Anabaena flos-aquae***
Exposure	**96 hour, static**
0-72-h E_rC_{50} - growth rate	**24.0 mg a.s./L (95% CI 21.6 to 26.6 mg a.s./L)**
0-96-h E_rC_{50} - growth rate	**38.3 mg a.s./L (95% CI 2.19 to 669.9 mg a.s./L)**
0-96-h E_bC_{50} - cumulative biomass	**20.9 mg a.s./L (95% CI 14.1 to 31.1 mg a.s./L)**

B. Observations

No physical abnormalities were observed in the controls or treatment groups during the study. The growth curves for the control and each test level demonstrate some inhibition of growth at the highest test concentration. The cells were observed for each test day during the cell counting procedure with no abnormal observations being noted.

CONCLUSION

The 72 and 96-hour growth rates were calculated based on initial measured concentrations. The 72 hour EC_{50} value for growth rate (E_rC_{50}) is calculated to be 24.0 mg a.s./L with LOEC and NOEC values of >21.7 and 21.7 mg a.s./L, respectively. The 96 hour E_rC_{50} value is 38.3 mg a.s./L with LOEC and NOEC values of >21.7 and 21.7 mg a.s./L, respectively.
The EC_{50} for growth rate is in excess of the highest dose tested and the practical limit of water solubility.

Effects of metabolites on algae

Report:	**KIIA 8.4/04, [redacted]; 2005**
Title:	*Pseudokirchneriella subcapitata* Growth Inhibition Test with BYI 08330-enol
	Date: 2005-06-26
Organisation:	[redacted], Germany
Report No.:	EBENM010, M-252331-01-2
Publication:	Unpublished
Dates of experimental work:	November 23, 2004 – December 16, 2004
Guidelines:	Draft Proposal for Updating OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (Feb. 18, 2004)
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* (freshwater microalgae, formerly known as *Selenastrum capricornutum*) expressed as NOEC, LOEC and ECx for growth rate of algal biomass (cells per volume). *Pseudokirchneriella subcapitata* were exposed in a chronic multigeneration test for 3 days under static exposure conditions to the nominal concentrations of 1.0, 3.1, 10, 31 and 100 mg p.m. (pure metabolite)/L in comparison to controls. Due to analytical measurements all results are based on nominal test concentrations.
The (0 - 72 h)-E_rC_{50} for BYI 08330-enol is >100 mg p.m./L and the (0 - 72 h)-NOE_rC is 31 mg p.m./L (based on nominal initial concentrations).

MATERIAL AND METHODS

A Materials

1. Test material	**BYI 08330-enol**
Description	**Beige powder**
Lot/batch No.	**Batch no.: 692-101-09-0005** **Tox no.: TOX 06850-00**
Purity	**99.1%**
Stability of test compound	**Expiration date: 2005-3-17 when stored at room temperature**
2. Vehicle and/or positive control	**Dimethylformamide (DMF) was used as solvent for the test item.**
3. Test animals	
Species	***Pseudokirchneriella subcapitata*, formerly named *Selenastrum capricornutum*, strain SAG 61.81**
Age	**Exponentially growing inoculum**
Source	**Collection of Algal Cultures, Inst. for Plant Physiology, University of Göttingen, Göttingen, Germany. Transferred to the laboratory on July 15, 2002 and kept since then.**
Acclimation period	**An inoculum pre-culture was prepared 2-4 days before the start of the test and cultivated under the same conditions as in the main test.**
Environmental conditions	
Temperature	**23 ± 2°C**
Photoperiod	**Continuous illumination, 4440 - 8880 lux (± 15%)**

B Study design and methods

1. In life dates **November 23, 2004 - November 26, 2004**

2. Experimental treatments

Once every week 200 µL of a 7-9 days old stock culture was transferred into a 250 mL cotton plugged Erlenmeyer flask containing 50 mL of nutrient medium. Stock cultures of algae were kept at 23 ± 2°C with 16 h light/day. All operations were conducted under sterile conditions to handle an axenic algae culture.

To ensure that the algae used as inoculum were exponentially growing, an inoculum pre-culture was prepared 2-4 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, adequate dilution of the pre-culture was done with nutrient medium.

The test vessels consisted of 300 mL Erlenmeyer flasks, filled with 150 mL nutrient medium and inoculated algae cells. They were placed on a tablet rotating 100 rpm to prevent sedimentation of the cells without additional aeration. The medium was

freshly prepared according to the mentioned test. The incubation temperature was 22.3°C to 23.5°C over the whole period of testing at a continuous illumination of 6579 lux.

The stock solution was prepared immediately prior to the test, it contained 1008.9 mg BYI 08330-enol with 5 mL DMF. The stock solution was well agitated on a magnetic stirrer for at least 1 minute before further use. An adequate amount of the stock solution was transferred to a dilution series with DMF to obtain the concentration levels used in the study.

The actual study included 3 replicate vessels per test level (6 replicate vessels per control). The range of test concentrations was selected based on pre experiments: 0 (control), 0 (solvent control), 1.0, 3.1, 10, 31, and 100 mg p.m./L (nominal initial).

The test duration accounted to 3 days, the pH values ranged from 7.8 to 8.4 in the controls.

3. Observations

Morphological examinations of cells by 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. The extinctions were determined at a wave length of 578 nm using a single-beam-photometer.

The temperature was determined by a continuous measurement in one additional incubated glass vessel filled with the same amount of de-ionised water as in the test vessels. The pH was measured at least at the beginning and at the end of the exposure period in all test levels and the controls.

For analytical verification samples were analysed for the actual concentration of BYI 08330-enol present in the test medium at all treatment levels and the controls on day 0 and day 3. At exposure termination, therefore the contents of all replicate vessels were combined. The limit of quantitation (LOQ) for the method used (in water by HPLC-UV) was 5 µg/L. The method was validated within the current study.

EC_X values and confidence intervals were calculated for the stated exposure period, using a commercial program (ToxRat Professional©). The LOEC determinations from the appropriate parameter (inhibition) were done using the ANOVA procedure ($p = <0.05$, one-sided) and properly selected multiple t-tests.

RESULTS AND DISCUSSION

A. Findings

Test conditions met all validity criteria biomass increased in the control by a factor of 71.6 within 3 days (required at least 16). The percent coefficient of variation of sectional growth rates from day 0-1, day 1-2, and day 2-3 in the controls did not exceed by more than 35% (should not exceed 35%). The percent coefficient of variation of the average growth rate in each control replicate did not exceed by more than 7%. The pH values in the controls did not increase by more than 1.5 units.

The analytical findings of BYI 08330-enol in the treatment levels found on day 0 were 94 to 104% of nominal (average 99.4%). On day 3 analytical findings of 94 to 102% of nominal (average 98.2%) were found. All results are based on nominal test concentrations.
The pH values ranged from 7.8 to 8.4 in the controls, temperature, oxygen-saturation and continuous illumination corresponded to the aspired values.

The static 72 hour algae growth inhibition test resulted in the following tabulated effects:

Nominal initial concentration [mg p.m./L]	Cell Number after 72 h (means) per mL	(0-72 h) Average Specific Growth Rate [$days^{-1}$]	Inhibition of Average Specific Growth Rate [%]	Doubling time of algae cells [days]
Pooled controls	716,000	1.423	-	0.487
1.0	745,000	1.437	-1.0	0.482
3.1	827,000	1.472	-3.4	0.471
10	705,000	1.416	0.5	0.490
31	670,000	1.401	1.5	0.495
100	409,000	1.237	13.0	0.560

test initiation with 10,000 cells/mL

CONCLUSION

The (0 - 72 h)-E_rC_{50} for BYI 08330-enol is >100 mg p.m./L and the (0 - 72 h)-NOE_rC is 31 mg p.m./L (based on nominal initial concentrations).

Report: **KIIA 8.4/05, [redacted]; 2006**
Title: 4-Methoxycyclohexanone, Alga, Growth inhibition test
Date: 2006-07-27
Organisation: Bayer Industry Services, Leverkusen, Germany
Report No.: 2006/0032/03, M-275954-01-1
Publication: Unpublished
Dates of experimental work: June 14, 2006 and July 28, 2006
Guidelines: EU Council Directive 92/69/EEC (1992), part C.3
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 *Desmodesmus subspicatus* (planktonic freshwater algae) expressed as NOEC, LOEC and EC_{50} for growth (increase in cell density) and growth rate of algal biomass (rate of increase in cell density with time). In a limit test, *Desmodesmus subspicatus* were exposed in a multigeneration test for 3 days under static exposure conditions to nominal concentrations of 0 and 100 mg p.m./L, corresponding to measured concentrations of < 0.05 and 92.1 – 93.9 mg p.m./L, respectively.
The pH values ranged from 8.1 to 8.6 in the control, the incubation temperature and the continuous illumination corresponded to the aspired values. Due to analytical measurements in

control and treatment groups on day 0 and day 3 of the exposure period, all results are given as nominal test concentrations of the test substance.
Both, the (0 - 72h)-E_rC_{50} and the (0 - 72h)-E_bC_{50}, for 4-Methoxycyclohexanone were determined to be > 100 mg p.m./L.

MATERIAL AND METHODS

A Materials

1. Test material	4-Methoxycyclohexanone
Description	Not stated
Lot/batch No.	Batch no.: SAV5506-001
Purity	97.9%
Stability of test compound	Not stated
2. Vehicle and/or positive control	No vehicle and/or positive control
3. Test animals	
Species	*Desmodesmus subspicatus* (freshwater algae), non-axenic strain
Age	Exponentially growing stock cultures
Source	Collection of Algal Cultures, Inst. for Plant Physiology, University of Göttingen, Germany, maintained in the test facility
Acclimation period	An inoculum pre-culture was prepared 3 days before the start of the test and cultivated under the same conditions as in the main test.
Environmental conditions	
Temperature	21 - 25°C (mean +/- 2°C)
Photoperiod	Continuous uniform illumination, 4000 - 8000 lux (measured in the range of 400 - 700 nm)

B Study design and methods

1. In life dates: June 17, 2006 - July 28, 2006
2. Experimental treatments

Stock cultures of algae were kept at 23 ± 2°C at a light intensity of 4000 to 8000 lux (measured in the range of 400 to 700 nm). The nutrient medium was renewed once a week. To ensure that the algae used as inoculum were exponentially growing, an inoculum pre-culture was prepared 3 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 5000 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 test vessels were 300 mL Erlenmeyer flasks with cotton stoppers, filled with nutrient medium and inoculated algal cells (initial cell density approx. 5000 cells per mL). The incubation temperature was 23 ± 2°C over the whole period of testing at continuous illumination.

A direct weighing was prepared to give the desired test concentration. To achieve this, 125.2 mg 4-Methoxycyclohexanone were added to 1 L of dilution water, treated with an ultra turrax for 60 sec at 8000 rpm and afterwards stirred for 1 hour on a magnetic stirrer.

The study included 3 replicate vessels per test level (6 replicate vessels per control). The tested concentration was 100 mg p.m./L (nominal). Additionally, the highest test concentration was incubated without algae. The test duration was 3 days.

3. Observations

Cell densities were measured in a microcell counter or, alternatively, are determined by means of a microscopic counting chamber.

In order to avoid an impairment of the test system, an additional replicate was used for analysis and pH measurement at the beginning of the test. Analysis and pH measurement at the end of the test were performed in normal replicates.

For analytical verification samples were analysed by GC-MS for the actual concentration of 4-Methoxycyclohexanone present in the test medium in the treatments and controls on day 0 and day 3.

EC_{50} values and confidence intervals were determined by probit analysis. If possible, NOEC and LOEC were determined by a multisample comparison (according to Dunnett).

RESULTS AND DISCUSSION

A. Findings

The test conditions met all validity criteria given by the mentioned guidelines.

The analytical findings of 4-Methoxycyclohexanone in the treatment levels on day 0 were 86.9 to 97.3% of nominal (average 92.1%). On day 3 analytical findings of 93.7 to 94.1% of nominal (average 93.9%) were determined. Therefore all reported results are based on nominal concentrations of the test item.

The pH values ranged from 8.1 to 8.6 in the controls and the incubation temperature was in the range of 23 ± 2°C over the whole period of testing at a continuous illumination of 4000 to 8000 lux.

The static 72 hour algae growth inhibition test resulted in the following tabulated effects:

Nominal test concentration [mg p.m./L]	Cell density (cells/mL) after 72 h (means)	(0-72h) Growth Rate (r) [days^{-1}]	Inhibition of specific growth rate [%]	(0-72h) Growth (b) [integral of biomass]	Inhibition of growth [%]
Control	405,556	1.47	-	267,222	-
100	466,667	1.51	-3.2	313,889	- 17.5

test initiation with 5000 cells/mL

B. Observations

Because of technical problems concerning the measuring device, the samples taken at test start for analysis were determined with a delay of 48 hours. The samples were stored cold in a refrigerator until determination.

CONCLUSION

Both, the (0 - 72h)-E_rC_{50} and the (0 - 72h)-E_bC_{50}, for 4-Methoxycyclohexanone were determined to be > 100 mg p.m./L.

IIA 8.5 Effects on sediment dwelling organisms

IIA 8.5.1 Acute test

The objective of the following acute (48 hour) toxicity tests is to evaluate the acute toxicity to larvae of *Chironomus riparius* (1st instar) caused by the test item. As primary endpoint, a concentration causing 50% mortality to larvae of *Chironomus riparius* (48 h LC_{50}) has to be determined.

For this purpose, different levels (concentrations) of the test item were prepared in a range of geometric concentrations in ELENDT-medium. Larvae of *Chironomus riparius* were exposed under defined conditions to such different test levels in a water-only study and compared against control(s). Beside mortality a possible occurrence of symptoms was recorded and evaluated after 48 hours of exposure.

Report:	**KIIA 8.5.1/01, [redacted], 2005**
Title:	Acute Toxicity of BYI 08330 (tech.) to Larvae of *Chironomus riparius* in a 48 h Water-only Study.
	Date: 2005-12-08
Organisation:	[redacted], Germany
Report No.:	EBFNX072; M-262632-01-2
Publication:	Unpublished
Dates of experimental work:	June 03, 2005 – July 13, 2005
Guidelines:	No specified guideline (study is performed according to general aspects as quoted under OECD -202 (1984) and the corresponding revised OECD draft proposal, dated February 01, 2004)
Deviations:	not applicable
GLP:	yes (certified laboratory)

Executive summary

The objective of this 48 hour toxicity test is to evaluate the acute toxicity to larvae of *Chironomus riparius* (1st instar) caused by the test item, spirotetramat (BYI 08330 tech.). As primary endpoint, a concentration causing 50% mortality to larvae of *Chironomus riparius* (48 h -LC_{50}) was determined. For this purpose, larvae of *Chironomus riparius* were exposed under defined conditions to the nominal concentrations 0.76, 1.00, 1.80, 3.20, 5.60 and 10.0 mg a.s./L and compared against controls. Besides mortality a possible occurrence of symptoms was recorded and evaluated after 48 hours of exposure.

The incubation temperature, the oxygen content and pH values corresponded to the aspired values.

Quantitative amounts of BYI 08330 were measured in all test levels and control on day 0 and at the end of exposure, on day 2. Due to the high recoveries at the beginning of the exposure (average 108%) and the analytical findings after 2 days (average 83%), all results are based on nominal initial concentrations of the active ingredient.

The 48 h - LC_{50} was calculated by Probit analysis to be 1.38 mg a.s./L (C.I. 95%: 1.19 – 1.60 mg a.s./L).

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**White powder**
Lot/batch No.	**Batch no.: Mix-batch 08045/0014; TOX no.: 06689-02**
Purity	**97.4%**
Stability of test compound	**Expiration date: 2005-11-23, when stored at room temperature**
2. Vehicle and/or positive control	**Acetone (0.100 mL/L)**
3. Test animals	
Species	***Chironomus riparius***
Age	**1st - instar-larvae, < 2-3 days old**
Source	**University of [redacted] (UK), transferred in autumn 1991 to the laboratory and kept since then**
Acclimation period	**Larvae were obtained by introducing some fresh egg masses in small dishes with test medium. Two to three days after hatching the identification of the species was confirmed using a stereo microscope, and the larvae were transferred carefully with a blunt pipette to the test vessels.**
Environmental conditions	
Temperature	**20 ± 2°C**
Photoperiod	**16 to 8 hours light-dark-cycle (light intensity approx. 500 - 1000 lux**

B Study design and methods

1. In life dates **June 3, 2005 - June 8, 2005**

2. Experimental treatments

To start a culture, 2 - 4 egg masses were placed into a basin, whose bottom was covered with a thin layer of "Kieselgur" (silica) and a 5 - 7cm high layer of gently aerated M7–medium according to Elendt (based on deionised water). The basin was situated in a cage, which had gauze on each side. The hatched larvae were fed with green algae and an aqueous suspension of a vegetable fish food (Tetra Phyll®). After two or three days the larvae were to be harvested or after 2 to 3 weeks the adults emerged. After mating, female adults laid egg masses on the water surface and starting of a culture possibly took place again.

The actual study included 4 replicate vessels per test level, control and solvent control additionally. The test vessels consisted of 100 mL glass beakers, filled with 25 mL freshly prepared M7-medium and 10 animals each. Only one time, directly after insertion of the larvae into the test vessels, a small amount of 0.01 mL of an

aqueous fish food suspension (50 g Tetra Phyll®/L deion. water) was added to each test beaker (corresponding to 20 mg fish food/L test solution).
The stock solution was prepared immediately prior to the test, it contained 1.0268 g BYI 08330 (tech.) in 10 mL acetone p.a. From this stock solution 1, 0.100 mL was given to 1000 mL test medium (M7) to obtain stock solution 2. The test concentrations were prepared by adding appropriate aliquots of stock solution 2 to the test medium to yield the nominal test concentrations used in this study.
The range of test concentrations was selected basing on pre-experiments or historical data: 0 (control), 0 (solvent control), 0.56, 1.00, 1.80, 3.20, 5.60 and 10.0 mg BYI 08330 (a.s.)/L test medium (water-only study), the test duration was 2 days.

3. Observations

The temperature, the oxygen content and the pH were measured at the beginning (day 0, before inserting the larvae) and the end of the exposure period (day 2) in one test vessel of each test concentration and the control.
At the end of the test, the number of dead larvae (animals showing no swimming movements within 15 seconds after slight agitation of the vessel) and additional observations for sub-lethal effects were recorded for each test vessel separately with a binocular. Significant features of the test medium (e.g. presence of undissolved material) were also noted.
For analytical verification samples were analysed for the actual concentration of BYI 08330 present in all freshly prepared test levels on day 0 (including controls), and in all aged test levels on day 2 at the end of the exposure period. For sampling of aged test media, the contents of all four replicate vessels on day 2 were combined. The limit of quantitation (LOQ) for the method used (in water by HPLC-UV) was 5 µg/L. The method was validated within the current study.
For LC_{50} values and confidence intervals were calculated for the exposure period, using a commercial program (ToxRat Professional®).

RESULTS AND DISCUSSION

A. Findings

Validity criteria were met as control mortality was not more than 10% (being 8.7% for pooled controls) within 48 hours and measured dissolved oxygen did not decline below 60% oxygen saturation in the control and in all test concentrations.
The incubation temperature ranged from 20.4°C to 21.0°C over the whole period of testing (light intensity on day 0: 848 lux). The pH values ranged from 8.2 to 8.9. So the physical and chemical characteristics showed no deviations from defined guideline recommendations.
The analytical findings of BYI 08330 found in all freshly prepared test levels on day 0 in reference to nominal concentrations ranged between 106 and 109% (average 108%). In aged test levels on day 2 analytical findings were between 82 and 83% (average 83%) of the nominal. Due to the high recoveries at the beginning of the exposure and the analytical findings after 2 days, all results are based on nominal initial concentrations of the active ingredient.

Acute toxicity of BYI 08330 to first instar-Larvae of *Chironomus riparius* after 48 hours (based on nominal initial concentrations):

Test Concentration (nominal) [mg a.s./L]	Exposed Chironomids (=100%)	Mortality after 48 h	
		n	%
Control	40	3	7.5
Solvent Control *	40	4	10.0
0.56	40	4	10.0
1.00	40	13	32.5
1.80	40	29	72.5
3.20	40	39	97.5
5.60	40	40	100.0
10.0	40	40	100.0

* acetone p.a. (0.100 mL/L)

CONCLUSION

Based on nominal initial concentrations of spirotetramat, the 48 h- LC_{50} was calculated by Probit analysis to be 1.38 mg a.s./L (C.I. 95%: 1.19 – 1.60 mg a.s./L).

Effects of metabolites on *Chironomus riparius*

Report:	**KIIA 8.5.1/02, [redacted]; 2005**
Title:	Acute Toxicity of BYI 08330-enol to Larvae of *Chironomus riparius* in a 48 h Water-only Study.
	Date: 2005-12-08
Organisation:	[redacted], Germany
Report No.:	EBFNX073; M-262638-01-2
Publication:	Unpublished
Dates of experimental work:	May 20, 2005 – June 14, 2005
Guidelines:	No specified guideline (study is performed according to general aspects as quoted under OECD -202 (1984) and the corresponding revised OECD draft proposal, dated February 01, 2004)
Deviations:	not applicable
GLP:	yes (certified laboratory)

Executive summary

The aim of this 48 hour test is to evaluate the acute toxicity to larvae of *Chironomus riparius* (1st instar) caused by the test item. As primary endpoint, a concentration causing 50% mortality to larvae of *Chironomus riparius* (48 h-LC_{50}) was determined. For this purpose, larvae of *Chironomus riparius* were exposed for 48 hours in a static test system to nominal initial concentrations of 1.00, 2.00, 4.00, 8.00, 16.0, 32.0 and 64.0 mg pure metabolite (p.m.)/L in a water-only study and compared against a control. Besides mortality a possible occurrence of symptoms was recorded and evaluated after 48 hours of exposure.

The incubation temperature, the oxygen content and pH values corresponded to the aspired values.

Quantitative amounts of BYI 08330-enol were measured in all freshly prepared test levels on day 0 and control. On day 2, at the end of exposure, all aged test levels including control were

analysed additionally. Due to the high recoveries at the beginning of the exposure (average 102%) and the analytical findings after 2 days (average 98%), all results are based on nominal initial concentrations of the pure metabolite.
The 48 h - LC_{50} was calculated by Probit analysis to be 74.9 mg p.m./L (C.I. 95%: 48.9 - 115).

MATERIAL AND METHODS

A Materials

1. Test material	**BYI 08330-enol**
Description	**Beige powder**
Lot/batch No.	**Batch no.: 692-101-09-0005**
	Tox no.: 06850-01
Purity	**99.1%**
Stability of test compound	**Expiration date: 2005-09-23, when stored at room temperature**
2. Vehicle and/or positive control	**Elendt M7-medium, based on de-ionised water, was used as test and culture medium.**
3. Test animals	
Species	***Chironomus riparius* (larvae)**
Age	**1st instars, < 2-3 days old**
Source	**University of [redacted] (UK); transferred in autumn 1991 and kept since then**
Acclimation period	**Larvae were obtained by introducing some fresh egg masses in small dishes with test medium. Two to three days after hatching the identification of the species was confirmed using a stereo microscope, and the larvae were transferred carefully with a blunt pipette to the test vessels.**
Environmental conditions	
Temperature	**20 ± 2°C**
Photoperiod	**16:8 hours (light to dark), 500-1000 lux**

B Study design and methods

1. In life dates	**May 20, 2005 – June 14, 2005**

2. Experimental treatments

For breeding the midges are kept in cages. A basin is set on the bottom of each cage. The bottom of the basins are covered with a thin layer of "Kieselgur" (silica) and a 5 - 7 cm high layer of reconstituted water M7 according to Elendt, which is aerated gently. To start a culture in the cage, 2 - 4 egg masses are placed into the prepared basin. The hatched larvae are fed with green algae and an aqueous suspension of a vegetable fish food (Tetra Phyll®). After 2 to 3 weeks the adults emerge. After mating, female adults will lay egg masses on the water surface where these can be taken to start a new culture or to perform toxicity tests.

The stock solution was prepared immediately prior to the test containing 100.7 mg BYI 08330-enol in 1000 mL test medium (M7). From this stock solution dilution series were made: 0 (control), 1.00, 2.00, 4.00, 8.00, 16.0, 32.0 and 64.0 mg BYI 08330-enol/L test medium (water-only study). The range of test concentrations was selected basing on pre-experiments or historical data.
Per test concentration and control 4 test vessels were established. The test vessels consisted of 100 mL glass beakers, each filled with 25 mL freshly prepared M7-medium and 10 animals. Only one time, directly after insertion of the larvae into the test vessels, a small amount of 0.01 mL of an aqueous fish food suspension (50 g Tetra Phyll®/L deion. water) was added to each test beaker containing 25 mL test solution (corresponding to 20 mg fish food/L test solution).

3. Observations

48 hours after test initiation, the number of dead larvae (=animals showing neither swimming movements within 15 seconds after slight agitation of the vessel) were recorded for each test vessel separately with a binocular. Additional observations for sub-lethal effects were performed and recorded for each test vessel separately. Significant features of the test medium (e.g. presence of undissolved material) were also noted.
Temperature, pH and dissolved oxygen were measured at the beginning (day 0, before inserting the larvae) and the end of exposure period (day 2) in one test vessels of each test concentration and the control.
For analytical verification, samples were analysed for the actual concentration of BYI 08330-enol present in all freshly prepared test levels on day 0 and control, and in all aged solutions on day 2 at the end of exposure period. Aliquots for freshly prepared test levels for day 0 analyses were sampled from the prepared volume of each test level. For sampling of aged test media, the contents of all four replicate vessels on day 2 were combined.
For LC_{50} values and confidence intervals were calculated for the exposure period, using a commercial program (ToxRat Professional®).

RESULTS AND DISCUSSION

A. Findings

All validity criteria were met as the control mortality did not exceed 10% and measured dissolved oxygen concentrations in the control and all test levels did not decline below 60% oxygen saturation during exposure. The incubation temperature ranged from 20.8°C to 21.0°C and the pH values from 7.8 to 8.4 over the whole period of testing.
The analytical findings of BYI 08330-enol found in all freshly prepared test levels on day 0 in reference to nominal concentrations ranged between 98 and 105% (average 102%). In aged test levels on day 2 there were analytical findings between 88 and 104% (average 98%) of nominal. Due to the high recoveries at the beginning of the exposure and the analytical findings after 2 days, all results are based on nominal initial concentrations of the pure metabolite.

Acute toxicity of BYI 08330-enol to first instar-larvae of *Chironomus riparius* after 48 hours (based on nominal initial concentrations):

Test Concentration (nominal) [mg p.m./L]	Exposed Chironomids (=100%)	Mortality after 48 h	
		n	%
Control	**40**	**0**	**0.0**
1.00	**40**	**0**	**0.0**
2.00	**40**	**0**	**0.0**
4.00	**40**	**0**	**0.0**
8.00	**40**	**0**	**0.0**
16.0	**40**	**5**	**12.5**
32.0	**40**	**11**	**27.5**
64.0	**40**	**16**	**40.0**

CONCLUSION

Based on nominal initial concentrations of the pure metabolite BYI 08330-enol the 48 h - LC_{50} was calculated by Probit analysis to be 74.9 mg p.m./L (C.I. 95%: 48.9 – 115).

Report: **KIIA 8.5.1/03, [redacted]; 2006**
Title: Acute Toxicity of BYI 08330-ketohydroxy to Larvae of *Chironomus riparius* in a 48 h Static Laboratory Test System.
Date: 2006-01-16
Organisation: [redacted], Germany
Report No.: EBFNO347; M-263956-01-2
Publication: Unpublished
Dates of experimental work: June 16, 2005 – June 22, 2005 (biological part)
Guidelines: No specified guideline (study is performed according to general aspects as quoted under OECD -202 (1984) and the corresponding revised OECD draft proposal, dated February 01, 2004)
Deviations: not applicable
GLP: yes (certified laboratory)

Executive summary

The objective of this 48 hour toxicity test is to evaluate the acute toxicity to larvae of *Chironomus riparius* (1st instar) caused by the test item, BYI 08330-ketohydroxy. As primary endpoint, a concentration causing 50% mortality to larvae of *Chironomus riparius* (48 h -LC_{50}) was determined. For this purpose, larvae of *Chironomus riparius* were exposed under defined conditions to the nominal concentrations 1.00, 3.60, 10.0, 36.0 and 100 mg pure metabolite (p.m.)/L and compared against a control. Besides mortality a possible occurrence of symptoms was recorded and evaluated after 48 hours of exposure.

The incubation temperature, the oxygen content and pH values corresponded to the aspired values.

Quantitative amounts of BYI 08330-ketohydroxy were measured in all test levels and control on day 0 and at the end of exposure, on day 2. Due to the high recoveries at the beginning of the

exposure (average 97%) and the analytical findings after 2 days (average 94%), all results are based on nominal concentrations of the pure metabolite.
The 48 h - LC_{50} was determined by Probit analysis to be > 100 mg p.m./L (calculation of confidence interval was not determinable due to mathematical reasons).

MATERIAL AND METHODS

A Materials

1. Test material	**BYI 08330-ketohydroxy**
Description	**Light yellow solid**
Lot/batch No.	**Batch no.: NLL7549-7** **TOX no.: 06867-00**
Purity	**98.6%**
Stability of test compound	**Expiration date: 2005-07-27, when stored at room temperature**
2. Vehicle and/or positive control	**None (water solubility 218 mg/L)**
3. Test animals	
Species	***Chironomus riparius***
Age	**1st- instar-larvae, < 2-3 days old**
Source	**University of [redacted] (UK), transferred in autumn 1991 to the laboratory and kept since then**
Acclimation period	**Larvae were obtained by introducing some fresh egg masses in small dishes with test medium. Two to three days after hatching the identification of the species was confirmed using a stereo microscope, and the larvae were transferred carefully with a blunt pipette to the test vessels.**
Environmental conditions	
Temperature	**20 ± 2°C**
Photoperiod	**16 to 8 hours light-dark-cycle (light intensity approx. 500 - 1000 lux**

B Study design and methods

1. In life dates **June 17, 2005 - June 22, 2005**

2. Experimental treatments

To start a culture, 2 - 4 egg masses were placed into a basin, whose bottom was covered with a thin layer of "Kieselgur" (silica) and a 5 - 7cm high layer of gently aerated M7-medium according to Elendt (based on deionised water). The basin was situated in a cage, which had gauze on each side. The hatched larvae were fed with green algae and an aqueous suspension of a vegetable fish food (Tetra Phyll®). After two or three days the larvae were to be harvested or after 2 to 3 weeks the adults

emerged. After mating, female adults laid egg masses on the water surface and starting of a culture possibly took place again.

The actual study included 4 replicate vessels per test level and control. The test vessels consisted of 100 mL glass beakers, filled with 25 mL freshly prepared M7-medium and 10 animals each. Only one time, directly after insertion of the larvae into the test vessels, a small amount of 0.01 mL of an aqueous fish food suspension (50 g Tetra Phyll®/L deion. water) was added to each test beaker (corresponding to 20 mg fish food/L test solution).

The stock solution was prepared immediately prior to the test, it contained 101.8 mg BYI 08330-ketohydroxy in 1000 mL test medium. The pH was adjusted near to 7 with phosphoric acid.

The range of test concentrations was selected basing on pre-experiments or historical data: 0 (control), 1.00, 3.60, 10.0, 36.0 and 100 mg BYI 08330-ketohydroxy/L test medium., the test duration was 2 days.

3. Observations

The temperature, the oxygen content and the pH were measured at the beginning (day 0, before inserting the larvae) and the end of the exposure period (day 2) in one test vessel of each test concentration and the control.

At the end of the test, the number of dead larvae (animals showing no swimming movements within 15 seconds after slight agitation of the vessel) and additional observations for sub-lethal effects were recorded for each test vessel separately with a binocular. Significant features of the test medium (e.g. presence of undissolved material) were also noted.

For analytical verification samples were analysed for the actual concentration of BYI 08330 present in all freshly prepared test levels on day 0 (including control), and in all aged test levels on day 2 at the end of exposure period (also including control). For sampling of aged test media, the contents of all four replicate vessels on day 2 were combined.

The limit of quantitation (LOQ) for the method used (in water by HPLC-UV) was 5 µg/L for BYI 08330-ketohydroxy. The method was validated within the current study.

For LC_{50} values and confidence intervals were calculated for the exposure period, using a commercial program (ToxRat Professional©).

RESULTS AND DISCUSSION

A. Findings

The physical and chemical characteristics showed no deviations from defined guideline recommendations. The incubation temperature ranged from 20.5°C to 21.6°C over the whole period of testing (light intensity at the beginning of the test 763 lux). The measured dissolved oxygen concentrations in the control and all test levels did not decline below 60% oxygen saturation during exposure. In contrast to the pre adjusted pH 7 of the stock solution and the test medium on day 0, the measured pH after preparing the test solutions was about 8 for all test solutions and the control. Due to the high analytical findings of the amounts of the test

item in test solutions after 2 days, the failed pH adjustment did not influence the results of the study negatively. The control mortality was not more than 10% within 48 hours (2.5%).
The analytical findings of BYI 08330-ketohydroxy found in all freshly prepared test levels on day 0 in reference to nominal concentrations ranged between 93 and 105% (average 97%). In aged test levels on day 2 analytical findings were between 92 and 96% (average 94%) of the nominal. Due to the high recoveries at the beginning of the exposure and the analytical findings after 2 days, all results are based on nominal initial concentrations of the pure metabolite.

Acute toxicity of BYI 08330-ketohydroxy to first instar-Larvae of *Chironomus riparius* after 48 hours (based on nominal initial concentrations):

Test Concentration (nominal) [mg p.m./L]	Exposed Chironomids (=100%)	Mortality after 48 h	
		n	%
Control	**40**	**1**	**2.5**
1.00	**40**	**2**	**5.0**
3.60	**40**	**1**	**2.5**
10.0	**40**	**1**	**2.5**
36.0	**40**	**2**	**5.0**
100.0	**40**	**11**	**27.5**

CONCLUSION

The 48 h - LC_{50} was determined to be > 100 mg p.m./L based on nominal initial concentrations (calculation of confidence interval was not determinable due to mathematical reasons).

Report: **KIIA 8.5.1/04, [redacted]; 2007**
Title: Acute Toxicity of BYI 08330-cis-methoxy-cyclohexylamino carboxylic acid to Larvae of *Chironomus riparius* in a 48 h Static Laboratory Test System (Limit-Test).
Date: 2007-04-27
Organisation: [redacted], Germany
Report No.: EBFNX005; M-287220-01-1
Publication: unpublished
Dates of experimental work: November 17, 2006 – December 18, 2006
Guidelines: No specified guideline
(study is performed according to general aspects as quoted under OECD Guideline No. 202 (Guideline for Testing of Chemicals, "Daphnia sp., Acute Immobilisation Test, adopted April 13, 2004"))
Deviations: not applicable
GLP: yes (certified laboratory)

Executive summary

The objective of this 48 hour toxicity test was to indicate, that the LC_{50} of the test item was above the limit concentration tested to larvae of *Chironomus riparius*.
For this purpose 1st instar larvae of *Chironomus riparius* (< 2-3 days old) were exposed to the limit concentration of 100 mg BYI 08330-cis-methoxy-cyclohexylamino carboxylic acid/L with

six replicates per test concentration and control, with 10 animals each, under defined exposure conditions in a static laboratory test system. Additionally to lethality the possible occurrence of symptoms was recorded and evaluated after 48 hours of exposure.
The analytical findings of BYI 08330-cis-methoxy-cyclohexylamino carboxylic acid found in the freshly prepared test level on day 0 in reference to nominal concentration was 87.8%. In the aged test level on day 2, 89.4% of nominal was found. Due to the high recovery at the beginning of the exposure and the analytical findings after 2 days, all results are based on nominal concentrations of the metabolite.
No sublethal effects were observed after 48 hours. No mortality was observed after 48 hours of static exposure for the control and the limit concentration, thus the LC_{50} for *Chironomus riparius* is greater than 100 mg BYI 08330-cis-methoxy-cyclohexylamino carboxylic acid/L.

MATERIAL AND METHODS

A Materials

1. Test material	**BYI 08330-cis-methoxy-cyclohexylamino carboxylic acid**
Description	**white powder**
Lot/batch No.	**batch-no.: KTS10330-1-4 Analysis ref.code: AZ 13712 LIMS-no.: 0624852**
Analysed content	**93.7%**
Stability of test compound	**Expiration date: 2008-09-13, when stored at -20 ± 5°C**
2. Vehicle and/or positive control	**No vehicle used. Reference substance: 3,5-dichlorophenol. Reference tests are done periodically (i.e. event driven in case of receiving new strains, introduction of new test conditions, apparatus, etc.). Respective documents were archived together with strain protocols.**
3. Test species	
Species	***Chironomus riparius***
Age	**1st instar-larvae, < 2-3 days old**
Source	**University of Frankfurt am Main (Germany), transferred in July 2006 to the laboratory and kept since then**
Acclimation period (Breeding conditions)	**First instar larvae (L1) of *Chironomus riparius*. Larvae were obtained by introducing some fresh egg masses in small dishes with test medium. Two to three days after hatching the identification of the species was confirmed using a stereo microscope, and the L1-larvae were transferred carefully with a blunt pipette to the test vessels.**
Environmental conditions	
Temperature	**20 ± 2°C**
Photoperiod	**16 to 8 hours light-dark-cycle (light intensity approx. 500 - 1000 lux**

B Study design and methods

1. In life dates **November 17, 2006 – December 18, 2006**

2. Experimental treatments

M7-medium, based on de-ionised water, was used as test medium. For preparation of the test solution 53.5 mg test item was added to 500 mL test medium (M7), prepared immediately prior to the test. The test solution was well agitated on a magnetic stirrer for at least 5 minutes before further use. The test concentration was selected based on pre-experiments as limit test at 100 mg/L. Exposure conditions were static for 2 days (48 hours).

The actual study included 6 replicate vessels per test level and control. The test vessels consisted of 100 mL glass beakers, filled with 25 mL freshly prepared M7-medium and 10 animals each. Once, directly after insertion of the larvae into the

test vessels, a small amount of 0.01 mL of an aqueous fish food suspension (50 g Tetra Phyll®/L deion. water) was added to each test beaker (corresponding to 20 mg fish food/L test solution).

3. Observations

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

Because all test vessels are placed under isothermal conditions, a continuous temperature measurement in one vessel was done to calculate the mean, min, and max figures for water temperature (based on continuously (hourly means) measured values). Temperature values are measured in one control beaker and recorded hourly by a data logger. Additionally the temperature was measured at the beginning (day 0, before inserting the larvae) and the end of exposure period (day 2) in one test vessel of each test concentration and the control with an electronic thermometer testo 112.

The pH was measured at the beginning (day 0, before inserting the larvae) and the end of exposure period (day 2) in one test vessel of each test concentration and the control. The pH was measured by an electronic Knick calimatic 766 pH meter.

Dissolved oxygen was measured at the beginning (day 0, before inserting the larvae) and the end of exposure period (day 2) in one test vessel of each test concentration and the control. Dissolved oxygen was measured by a WTW oxygen meter model 537.

Samples were analysed for the actual concentration of BYI 08330-cis-methoxy-cyclohexylamino carboxylic acid present in the freshly prepared test level on day 0 (including control), and in the aged test level on day 2 at the end of exposure period including control. Aliquots for freshly prepared test levels for day 0 analyses were sampled from the prepared volume of each test treatment level. For sampling of aged test media, the contents of all six replicate vessels on day 2 were combined, and the temperature, pH and dissolved oxygen was measured. The combined test solutions were then submitted for analyses. The limit of quantitation (LOQ) for the method used (in water by HPLC-MS/MS) was 0.8 µg/L. The method was validated within the current study.

RESULTS AND DISCUSSION

A. Findings

All validity criteria were met as control mortality was below 10% (being 0%) within 48 hours and measured dissolved oxygen was above 60% oxygen saturation in the control and in the test concentration.

The measured light intensity on day 0 was 648 lux. The measured temperature ranged from 20.3 to 20.9°C over the whole period of testing. The pH values ranged from 6.9 to 7.7 for the test item.

The analytical findings of BYI 08330-cis-methoxy-cyclohexylamino-carboxylic acid found in the freshly prepared test level on day 0 in reference to nominal concentration was 87.8%. In the aged test level on day 2, 89.4% of nominal was found. Due to the high recovery at the beginning of the exposure and the analytical findings after 2 days, all results are based on nominal concentrations of the metabolite.

Acute toxicity of test item to first instar-Larvae of *Chironomus riparius* after 48 hours (based on nominal concentration):

Test Concentration (nominal) [mg p.m./L]	Exposed Chironomids (=100%)	Mortality after 48 h	
		n	%
Control	60	0	0
100	60	0	0

B. Observations

No mortality was evaluated after 48 hours of static exposure for the control and the limit concentration. Observations on sublethal effects revealed no abnormal behaviour of the exposed larvae of *Chironomus riparius* after 48 hours.

CONCLUSIONS

No mortality was observed after 48 hours of static exposure for the control and the limit concentration, thus the LC_{50} for *Chironomus riparius* is greater than 100 mg BYI 08330-cis-methoxy-cyclohexylamino carboxylic acid/L.

Report:	**KIIA 8.5.1/05, [redacted]; 2007**
Title:	Acute Toxicity of 4-Methoxycyclohexanon to Larvae of *Chironomus riparius* in a 48 h Static Laboratory Test System (Limit-Test) Date: 2007-04-27
Organisation:	[redacted], Germany
Report No.:	EBFNX104; M-287218-01-1
Publication:	unpublished
Dates of experimental work:	November 17, 2006 – December 18, 2006
Guidelines:	No specified guideline (study is performed according to general aspects as quoted under OECD Guideline No. 202 (Guideline for Testing of Chemicals, "Daphnia sp., Acute Immobilisation Test, adopted April 13, 2004"))
Deviations:	not applicable
GLP:	yes (certified laboratory)

Executive summary

The objective of this 48 hour toxicity test was to indicate, that the LC_{50} of the test item was above the limit concentration tested to larvae of *Chironomus riparius*.
For this purpose 1st instar larvae of *Chironomus riparius* (< 2-3 days old) were exposed to the limit concentration of 100 mg 4-Methoxycyclohexanon/L with six replicates per test concentration and control, with 10 animals each, under defined exposure conditions in a static laboratory test system. Additionally to lethality the possible occurrence of symptoms was recorded and evaluated after 48 hours of exposure.
The analytical findings of 4-Methoxycyclohexanon found in the freshly prepared test level on day 0 in reference to nominal concentration was 109%. In the aged test level on day 2, 112% of nominal was found. Due to the high recovery at the beginning of the exposure and the analytical findings after 2 days, all results are based on nominal concentrations of the metabolite.
No sublethal effects were observed after 48 hours. No mortality was observed after 48 hours of static exposure for the control and the limit concentration, thus the LC_{50} for *Chironomus riparius* is greater than 100 mg 4-Methoxycyclohexanon/L.

MATERIAL AND METHODS

A Materials

1. Test material	**4-Methoxycyclohexanon**
Description	**colourless liquid**
Lot/batch No.	**LIMS No.: PBF-2006-0056-TOX-07505** **batch-no.: SAV5506-001** **development No.: 0255249** **article-No.: 05486343**
Analysed content	**97.9%**
Stability of test compound	**Expiration date: 2007-01-03, when stored at room temperature**
2. Vehicle and/or positive control	**No vehicle used.** **Reference substance: 3,5-dichlorophenol.** **Reference tests are done periodically (i.e. event driven in case of receiving new strains, introduction of new test conditions, apparatus, etc.). Respective documents were archived together with strain protocols.**
3. Test species	
Species	***Chironomus riparius***
Age	**1st instar-larvae, < 2-3 days old**
Source	**University of Frankfurt am Main (Germany), transferred in July 2006 to the laboratory and kept since then**
Acclimation period (Breeding conditions)	**First instar larvae (L1) of *Chironomus riparius*. Larvae were obtained by introducing some fresh egg masses in small dishes with test medium. Two to three days after hatching the identification of the species was confirmed using a stereo microscope, and the L1-larvae were transferred carefully with a blunt pipette to the test vessels.**
Environmental conditions	
Temperature	**20 ± 2°C**
Photoperiod	**16 to 8 hours light-dark-cycle (light intensity approx. 500 - 1000 lux**

B Study design and methods

1. In life dates: November 17, 2006 – December 18, 2006

2. Experimental treatments

M7-medium, based on de-ionised water, was used as test medium. For preparation of the test solution 51.3 mg test item was added to 500 mL test medium (M7), prepared immediately prior to the test. The test solution was well agitated on a magnetic stirrer for at least 5 minutes before further use. The test concentration was selected based on pre-experiments as limit test at 100 mg/L. Exposure conditions were static for 2 days (48 hours).

The actual study included 6 replicate vessels per test level and control. The test vessels consisted of 100 mL glass beakers, filled with 25 mL freshly prepared M7-medium and 10 animals each. Once, directly after insertion of the larvae into the

test vessels, a small amount of 0.01 mL of an aqueous fish food suspension (50 g Tetra Phyll®/L deion. water) was added to each test beaker (corresponding to 20 mg fish food/L test solution).

3. Observations

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

Because all test vessels are placed under isothermal conditions, a continuous temperature measurement in one vessel was done to calculate the mean, min, and max figures for water temperature (based on continuously (hourly means) measured values). Temperature values are measured in one control beaker and recorded hourly by a data logger. Additionally the temperature was measured at the beginning (day 0, before inserting the larvae) and the end of exposure period (day 2) in one test vessel of each test concentration and the control with an electronic thermometer test 112.

The pH was measured at the beginning (day 0, before inserting the larvae) and the end of exposure period (day 2) in one test vessel of each test concentration and the control. The pH was measured by an electronic Knick calimatic 766 pH meter.

Dissolved oxygen was measured at the beginning (day 0, before inserting the larvae) and the end of exposure period (day 2) in one test vessel of each test concentration and the control. Dissolved oxygen was measured by a WTW oxygen meter model 537.

Samples were analysed for the actual concentration of 4-Methoxycyclohexanon present in the freshly prepared test level on day 0 (including control), and in the aged test level on day 2 at the end of exposure period including control. Aliquots for freshly prepared test levels for day 0 analyses were sampled from the prepared volume of each test treatment level. For sampling of aged test media, the contents of all six replicate vessels on day 2 were combined, and the temperature, pH and dissolved oxygen was measured. The combined test solutions were then submitted for analyses. The limit of quantitation (LOQ) for the method used (in water by HPLC-MS/MS) was 1 µg/L. The method was validated within the current study.

RESULTS AND DISCUSSION

A. Findings

All validity criteria were met as control mortality was below 10% (being 0%) within 48 hours and measured dissolved oxygen was above 60% oxygen saturation in the control and in the test concentration.

The measured light intensity on day 0 was 648 lux. The measured temperature ranged from 20.3 to 20.9°C over the whole period of testing. The pH value was 8.0.

The analytical findings of 4-Methoxycyclohexanon found in the freshly prepared test level on day 0 in reference to nominal concentration was 109%. In the aged test level on day 2, 112% of nominal was found. Due to the high recovery at the beginning of the exposure and the analytical findings after 2 days, all results are based on nominal concentrations of the metabolite.

Acute toxicity of test item to first instar-Larvae of *Chironomus riparius* after 48 hours (based on nominal concentration):

Test Concentration (nominal) [mg p.m./L]	Exposed Chironomids (=100%)	Mortality after 48 h n	Mortality after 48 h %
Control	60	0	0
100	60	0	0

B. Observations

No mortality was evaluated after 48 hours of static exposure for the control and the limit concentration. Observations on sublethal effects revealed no abnormal behaviour of the exposed larvae of *Chironomus riparius* after 48 hours.

CONCLUSIONS

No mortality was observed after 48 hours of static exposure for the control and the limit concentration, thus the LC_{50} for *Chironomus riparius* is greater than 100 mg 4-Methoxycyclohexanon/L.

IIA 8.5.2 Chronic test

Report:	**KIIA 8.5.2/01, [redacted]; 2005**
Title:	*Chironomus riparius* 28-day Chronic Toxicity Test with BYI 08330 (tech.) in a Water-Sediment System using Spiked Water.
	Date: 2005-03-16
Organisation:	[redacted], Germany
Report No.:	EBFN0050; M-248099-02-2
Publication:	Unpublished
Dates of experimental work:	September 07, 2004 – December 17, 2004
Guidelines:	Proposal for a new OECD Guideline 219: "Sediment-Water Chironomid Toxicity Test Using Spiked Water" (April 2003)
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the influence of the test item (spirotetramat) on emergence and development of *Chironomus riparius* for 28-days in a static water-sediment-system (spiked water exposure), expressed as NOEC, LOEC and EC_x for emergence ratio and development rate. First instar of *Chironomus riparius* larvae were exposed in a static test system for 28 days to initial nominal concentrations in the overlying medium (spiked water application) of 0.05, 0.10, 0.20, 0.40, 0.80, 1.60 and 3.20 mg a.s./L of a water-sediment-system.

The incubation temperature, the oxygen content and pH values corresponded to the aspired values.
Emergence ratio and development time in the controls fulfilled the guideline requirements as well.
Chemical analysis of overlying water and pore water over time reflect expected aquatic fate data with high recoveries of 92.2% to 115.8% (mean 102.7%) at the beginning of the exposure period in the overlying water (day 0). Therefore, initial nominal concentrations of the active ingredient in overlying water were used for reporting and evaluation of the results. In the pore water of the sediment, only at the highest test concentration, very low concentrations of the testing item ranging from 0.03 to 0.1% nominal were detected.
For emergence ratio the EC_{15} was determined to be 0.27 mg a.s./L, the EC_{50} to be 0.46 mg a.s./L, the NOEC to be 0.1 mg a.s./L and the LOEC to be 0.2 mg a.s./L. For development rate, the EC_{15} and the EC_{50} value were higher than 0.80 mg a.s./L, the NOEC was determined to be 0.8 mg a.s./L and the LOEC to be 1.6 mg/a.s./L (based on nominal initial concentration).

MATERIAL AND METHODS

A Materials

1. Test material — **Spirotetramat (BYI 08330) tech.**

Description	**White powder**
Lot/batch No.	**Batch no.: Mix-batch 08045/0014** **TOX no.: 06689-00**
Purity	**97.2% (w/w)**
Stability of test compound	**Expiration date: 2004-11-04, when stored at room temperature**

2. Vehicle and/or positive control — **Acetone**

3. Test animals

Species	***Chironomus riparius***
Age	**1st instar-larvae, < 2-3 days old**
Source	**University of [redacted] (UK), transferred in autumn 1991 to the laboratory and kept since then**
Acclimation period	**The test vessels were prepared (filled with sediment and medium) 7 days before the study commenced.** **Larvae were obtained by introducing some fresh egg masses in small dishes with test medium. Two to three days after hatching the identification of the species was confirmed using a stereo microscope, and the L1-larvae were transferred carefully one day prior to treatment (= day -1) to the test vessels. Test vessels were aerated during the equilibration phase. The aeration of the water was stopped for 24 hours**

	after insertion of test organisms and re-started just before application of the test item.
Environmental conditions	
Temperature	20 ± 2°C
Photoperiod	16 to 8 hours light-dark-cycle (light intensity approx. 500 - 1000 lux

B Study design and methods

1. In life dates September 7, 2004 - October 12, 2004

2. Experimental treatments

In order to cultivate the test organisms, 2 - 4 egg masses were placed into a basin, whose bottom was covered with a thin layer of "Kieselgur" (silica) and a 5 - 7cm high layer of gently aerated M7-medium according to Elendt (based on deionised water). The basin was situated in a cage, which had gauze on each side. The hatched larvae were fed with green algae and an aqueous suspension of a vegetable fish food (Tetra Phyll®). After two or three days the L1-larvae were to be harvested or after 2 to 3 weeks the adults emerged. After mating, female adults laid egg masses on the water surface and starting of a culture possibly took place again.

The sediment in the sediment-water-system was made artificially. It was prepared 8 days before the start of the exposure period. It consisted of 74% fine quartz sand (68.2% of the sand has a particle size of 0.05 - 0.2mm), 4 - 5% dried, finely ground peat (sphagnum peat; pH 2 - 4), 20% kaolin (kaolinite content of about 56%, pH value approx. 7) and around 1% calcium carbonate to adjust the pH value to 7 ± 0.5 (figures refer to dry weight).

The sediment-water-system consisted of 0.6 L glass beakers. The bottom of these test vessels were covered with a 1.5 cm layer of wet sediment. M7-medium was added carefully to the sediment layer (for this short time, the sediment was covered by a sheet). The finally test water volume was 0.38 L, the height of the water column was 6.0 cm. Typically once a week the test vessels were refilled with deionised water up to the mark, which indicates the volume of 0.38 L overlying water in case of a loss of water by evaporation.

4 replicates each with 20 chironomids were applied for biological evaluations, additional replicates (with chironomids) were used for chemical analysis of the test item on day 0 and day 7 (for the control: 1 replicate and for highest, medium, and lowest test level: 2 replicates). For the chemical analysis on day 28 one beaker of the four beakers for biological evaluations was used. A further replicate of each test concentration was prepared with chironomids to measure the temperature, pH and oxygen content in the test water during the study.

To obtain the stock solution 0.3296 g test substance were dissolved in 10 mL acetone and several dilutions in acetone were made to obtain the application solutions for each test concentration. Appropriate amounts of the application solutions were applied carefully into the overlying water column of the vessels as a singular event on study day 0 by gently mixing of the water body.

Test vessels were aerated during the equilibration phase. The aeration of the water was stopped for 24 hours after insertion of test organisms and re-started just before application of the test item. Gentle aeration was provided through a glass Pasteur pipette situated about 2.5 cm above the sediment layer throughout the complete study over 28 days (approximately 2 bubbles per second). Test beakers were covered by clear plastic plates, preventing evaporation.

During the study the larvae were fed at least about three times per week with a commercial ornamental fish food extract (trade name Tetra Phyll®) as used for the breeding. An appropriate amount of this suspension (about 0.5 - 1 mg Tetraphyll®/Larvae/day) was added to each test container..

The range of test concentrations was selected in order to define the NOEC, LOEC and ECx; 0 (control), 0 (solvent control), 0.05, 0.10, 0.20, 0.40, 0.80, 1.60 and 3.20 mg a.s./L (nominal initial concentration in the overlying water), the test duration was 28 days.

3. Observations

The following physical-chemical parameters were measured in the overlying water of all additional test vessels: Once a week the temperature was measured; the pH was measured one day prior to the start of the study and once a week thereafter. Dissolved oxygen was measured twice per week. All parameters were additionally determined at the end of the test (day 28). Measurements of total hardness and of ammonia of the control and the highest test concentration (water phase) were performed on day 0 and day 28.

The test vessels were observed at least three times per week to make a visual assessment of any behavioural differences compared to the control. The sex, time point of emergence and number of emerged midges was recorded daily during the period of emergence.

The concentration of the active ingredient BYI 08330 was analysed in the overlying water column and the pore water of the sediment at three times during the study: 1 hour, 7 days and 28 days after application. The overlying water layer of the test containers was decanted carefully. Wet sediments of each beaker were filtered by vacuum (glass micro fibre filter, mesh size 1.0 µm), and the filtrates (= pore waters) were analysed. In addition, the application solution, the overlying water and pore water of the control and solvent control were also analysed on day 0.

The limit of quantitation (LOQ) for the method used (in water by HPLC-UV) was 5 µg/L. The method was validated within the current study.

The sensitivity differences between sexes are statistically judged by a Chi^2-r x 2 table test. EC_X values 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 (ToxRat Professional©). The LOEC determinations from the appropriate parameter (inhibition) were done using the ANOVA procedure ($p = <0.05$, one sided) and properly selected multiple t-tests.

RESULTS AND DISCUSSION

A. Findings

Test conditions met all validity criteria given by the mentioned guideline (emergence in the control(s) had to reach at least 70% of introduced larvae at the end of the test; emergence should occur between 12 and 23 days after their insertion into the control vessels; oxygen content in the water body had to be > 60% of saturation at the end of the test in all test vessels; pH of the overlying water had to be between 6 and 9 in all test vessels and water temperature had not to differ by more than ± 1°C over the whole exposure period.

The measured values for temperature, pH (water), dissolved oxygen, total hardness and ammonia ranged within typical tolerances of calibrated measuring devices and showed no deviations from defined guideline recommendations.

Dissolved oxygen concentrations ranged in the water phase from 6.9 to 8.5 mg O_2/L (7.2 mg O_2/L= 81% O_2 - saturation), the water pH values ranged from 8.3 to 8.7 and the water temperature ranged from 19.8°C to 20.1°C over the whole period of testing.

Chemical analysis of overlying water and pore water over time reflect expected aquatic fate data with high recoveries of 92.2% to 115.8% (mean 102.7%) at the beginning of the exposure period in the overlying water (day 0). Therefore, initial nominal concentrations were used for reporting and evaluation of the results. In the pore water of the sediment only at the highest test concentration very low concentrations of the testing item ranging from 0.03 to 0.1% nominal were detected.

Analytical results of BYI 08330:
average % of test concentrations of 0.05, 0.40 and 3.20 mg a.s./L

	1 hour / day 0	day 7	day 28
overlying water	**102.7**	**0.6**	**0**
pore water	**0.1**	**0.03**	**0**

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

Initial nominal Concentration (overlying water) [mg a.s./L]	Number of emerged midges	Emergence of inserted larvae			Development rate (1/d) Pooled sex
		total [%]	male [%]	female [%]	
controls (pooled)	144	90.0	43.1	46.9	0.061
0.05	65	72.3	42.50	38.75	0.063
0.10	72	90.0	40.00	50.00	0.062
0.20	59	74.8	42.50	31.25	0.062
0.40	54	67.5	36.25	31.25	0.062
0.80	6	7.5	3.75	3.75	0.061
1.60	0	0	-	-	-
3.20	0	0	-	-	-

The Chi²-Test indicated no statistically difference in sensitivities of sexes. Therefore male and female results were pooled for further statistical analyses of the development rate.
Statistical significance ($\alpha = 0.05$) on emergence ratio was evaluated for test concentrations of 0.20 to 0.80 mg a.s./L, resulting in an NOEC of 0.10 mg a.s./L. For the development rate (as determined by emergence) of pooled sex, there was no statistical difference up to the highest test concentration of 0.80 mg a.s./L. Nevertheless the NOEC and the LOEC are stated to be 0.80 and 1.60 mg a.s./L, respectively.

B. Observations

Start of emergence was on day 15 for the control and day 14 to 15 for all test concentrations from 0.05 to 0.80 mg a.s./L. No emergence could be observed at test concentrations of 1.60 and 3.20 mg a.s./L.
Abnormal observations (e.g. dead larvae or pupae, which failed to show full development and to emerge) throughout the study were observed at test concentration of 0.20 mg a.s. /L (one midge failed to emerge on day 25) and at 0.80 mg a.s./L (one midge failed to emerge on day 18). A treatment related effect seems to be not likely.

CONCLUSIONS

For emergence ratio the EC_{15} was determined to be 0.27 mg a.s./L and the EC_{50} to be 0.46 mg a.s./L. For development rate, the EC_{15} and the EC_{50} value were higher than 0.80 mg a.s./L.

IIA 8.6 Effects on aquatic plants

Report:	**KIIA 8.6/01, [redacted]; 2005**
Title:	*Lemna gibba* G3 - Growth Inhibition Test with BYI 08330 (tech.) under Static-renewal Test Conditions Date: 2005-07-29
Organisation:	[redacted], Germany
Report No.:	DOM 24019; M-255296-01-2
Publication:	Unpublished
Dates of experimental work:	February 13, 2004 - December 12, 2004
Guidelines:	OECD 221 "Lemna sp. Growth Inhibition Test" Revised Proposal for a New Guideline (February 2004)
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 *Lemna gibba* G3 expressed as NOEC, LOEC and EC*x* for growth rate of both response variables, frond number and total frond area of plants. The same endpoints are also determined for yield based on frond numbers and plant dry weight. 3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static renewal exposure conditions to the nominal concentrations of 0.30, 0.95, 3.05, 9.77, 31.3 and 100 mg a.s./L in comparison to controls. Quantitative amounts of BYI 08330 were measured in all freshly prepared test levels on day 0, 3, and 5 and additionally in all aged test levels on day 3, 5, and 7 of the exposure period.

The most sensitive response variable for the growth rate calculation is total frond area of plants resulting in an overall E_rC_{50} of 6.21 mg a.s./L. The lowest NOEC (0.47 mg a.s./L) is based on visual effects. For the yield calculation, the E_yC_{50} (frond number as the most sensitive response for the yield calculation) is 4.62 mg a.s./L.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.** **(synonym: AE 1302943; FHN 8330)**
Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014 (mix-batch)** **Tox no.: TOX 06689-00**
Purity	**97.2%**
Stability of test compound	**Approved until 2004-11-04 when stored at room temperature** **Hydrolytical stability: pH 4, pH 7: > 500 h (at 50°C), pH 9: 4 h (at 50°C)**
2. Vehicle and/or positive control	**Acetone was used as solvent for the test item.**
3. Test animals	
Species	***Lemna gibba*, strain G3**

Age	**7-10 days old colonies**
Source	**Dr. [redacted], Horticulture Crops Quality Laboratory, U.S. Department of Agriculture, Beltsville, U.S.A.**
	The plant material was transferred to the laboratory on June 27, 2002 and kept since then.
Acclimation period	**Stock cultures are maintained in glass dishes filled with growth medium under illumination of 6.50 - 10.0 klux and temperature of 24 ± 2°C for a minimum of three weeks. Transfers are made regularly into fresh medium to provide 7-10 days old colonies as test inoculum. All operations were conducted under sterile conditions to handle an axenic algae culture.**
Environmental conditions	
Temperature	**24 ± 2°C**
Photoperiod	**Permanent light; nominally 6.50-10.0 klux (± 15% variation from the mean).**

B Study design and methods

1. In life dates **February 13, 2004 - October 25, 2004**

2. Experimental treatments

Test vessels consisted of glass dishes with a diameter of 10 cm and a height of 6 cm (total volume of approx. 470 mL). They were covered with lids of glass to permit gas exchange and illumination under sterile conditions to the greatest possible extent. All dishes were placed in a growth incubator. In total, 3 replicate vessels per test level, 3 replicate vessels per control level, and 3 replicates at the solvent control level were established.

The growth medium (20X –AAP) was freshly prepared according to the mentioned test.

The stock solution was prepared immediately prior to the test and all renewals of the test media using 10288.1 mg test item and 50 mL acetone. It was well agitated on a magnetic stirrer for at least 1 minute before further use. From this stock solution dilution series (dilutions steps: 1/3.2) were made. The test concentrations were prepared by adding appropriate aliquots of these stock solutions to the test medium to yield the nominal test concentrations used in this study.

The range of test concentrations was selected based on pre experiments in order to define the NOE_rC, LOE_rC and E_rC_x (to cover preferably the range up to 75% growth rate inhibition), resulting in definitive concentrations of nominal 0 (control), 0 (solvent control), 0.30, 0.95, 3.05, 9.77, 31.3, and 100 mg a.s./L. The data was also used to define the US EPA specific endpoints for yield (NOE_yC, LOE_yC and E_yC_x. The test duration accounted to 7 days under static renewal conditions (based on hydrolytical instability under alkaline conditions).

3. Observations

Observations of changes in plant development were made on study days 3, 5, and 7 (e.g. frond size, appearance, necrosis, chlorosis or gibbosity, colony break-up or loss of buoyancy, root length, morphology or breakdown). Frond area, frond numbers and plant dry weights were determined.

Temperature data was recorded hourly by a data logger, pH was measured in all freshly prepared and all aged test levels and the controls, light was measured at least once.

Test item samples were analysed for the actual concentration of BYI 08330 present in all freshly prepared test levels on day 0, 3, and 5 and in all aged test levels on day 3, 5, and 7 of the exposure period.

Counting of fronds and determination of total frond area were carried out using the LemnaTec Scanalyzer machine, validated for such measurements. Dry weight of plants (needed for US-EPA-typical yield calculations) was determined on day 7.

ECx values and confidence intervals were calculated for the stated exposure period, the LOEC determinations from the appropriate parameter (inhibition) were done using the ANOVA procedure ($\alpha = 0.05$, one sided) and properly selected multiple t-tests. All statistical evaluations were done using the commercial program ToxRat Professional.

RESULTS AND DISCUSSION

A. Findings

Test conditions met the validity criteria given by the mentioned guideline as the frond number in the controls increased by a factor of 9.3 within 7 days corresponding to a doubling time (Td) of about 2.2 days.

The pH values ranged from 7.3 to 8.7 in the controls and the incubation temperature ranged from 23.5°C to 24.8°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 8040 lux.

The analytical findings of spirotetramat found in all freshly prepared test levels on day 0, 3, and 5 in reference to nominal concentrations ranged between 45 and 114% (average 78%). In aged test levels on days 3, 5 and 7 there were analytical findings between 19 and 64% (average 46%) of nominal. Therefore all results are based on the time weighted means of the measured concentrations of BYI 08330.

The test level 100 mg a.s./L was excluded from EC_{50}-calculations because of exceeded water solubility.

The static renewal 7 day growth inhibition test provided the following tabulated effects:

Nominal test levels [mg a.s./L] (time weighted mean measured mg a.s./L)	Final frond no. (replicate means, day 7)	Total frond area of plants (replicate means) [mm^2]	% inhibition of growth rate*	
			average growth rate for frond no.	average growth rate for total frond area of plants
control	112	914	--	--
Solvent control	118	992		
Pooled controls	115	953	--	--
0.30 (0.14)	108	1031	2.4	-3.6
0.95 (0.47)	100	992	5.8	-4.0
3.05 (1.54)	101	969	5.7	-2.7
9.77 (6.06)	54	311	34.0	50.1
31.3 (20.5)	24	121	70.2	91.8
100 (71.4) **	9	72	113.5	116.1

* negative value means growth stimulation

** excluded from EC_{50}-calculations because of exceeded water solubility

Nominal test levels [mg a.s./L] (time weighted mean measured mg a.s./L)	Final frond no. (replicate means, day 7)	Net weight (g) day 7 less inoculum net weight at day 0	% inhibition of yield*	
			Based on frond no.	Based on dry weight
Control	112	13	--	--
Solvent control	118	14.5	--	--
Pooled controls	115	12.8		--
0.30 (0.14)	108	14.2	6.5	-3.5
0.95 (0.47)	100	14.5	14.2	-5.5
3.05 (1.54)	101	14.1	13.9	-2.3
9.77 (6.06)	54	7.5	59.5	45.7
31.3 (20.5)	24	1.7	88.7	87.4
100 (71.4) **	9	0.3	102.9	97.6

* negative value means growth stimulation

** excluded from EC_{50}-calculations because of exceeded water solubility

B. Observations

Observed visual effects:

Nominal test level in mg a.s./L (time weighted mean measured mg a.s./L)	Visual effects
Control Solvent control 0.30 (0.14) 0.95 (0.47)	no visual effects observed
3.05 (1.54)	slight chlorosis on day 7
9.77 (6.06)	strong chlorosis on days 5 and 7; medium necrosis on day 7
31.3 (20.5)	strong necrosis and strong chlorosis on days 5 and 7
100 (71.4) **	test substance on the bottom in freshly prepared and aged test level; slight necrosis on day 3, strong necrosis and strong chlorosis on day 5; plants lethal on day 7

** excluded from EC_{50}-calculations because of exceeded water solubility

CONCLUSION

Results are based on the time weighted mean of the measured concentrations of the test item (test level 100 mg a.s./L excluded).

Growth rate

Endpoint (0-7 day)	Effect on frond no. [mg a.s./L]	Effect on total frond area of plants [mg a.s./L]
E_rC_{50} (CI 95%)	10.4 (7.96 – 13.8)	6.21 (5.33 – 7.18)
LOE_rC	6.06	6.06
NOE_rC	1.54	1.54

Yield

Endpoint (0-7 day)	Effect on frond no. [mg a.s./L]	Effect on plant weight [mg a.s./L]
E_yC_{50} (CI 95%)	4.62 (2.04-10.3)	6.96 (5.76-8.38)
LOE_yC	6.06	6.06
NOE_yC	1.54	1.54

The most sensitive response variable for growth rate is total frond area of plants resulting in an overall E_rC_{50} of 6.21 mg a.s./L. The most sensitive response variable for yield is frond number which results in a E_yC_{50} of 4.62 mg a.s./L. The lowest NOEC (0.47 mg a.s./L) is based on visual effects.

Effects of metabolites on aquatic plants

Report:	**KIIA 8.6/02, [redacted]; 2005**
Title:	*Lemna gibba* G3 - Growth Inhibition Test with BYI 08330-enol under Static Conditions Date: 2005-06-07
Organisation:	[redacted], Germany
Report No.:	EBFNX065; M-252412-01-2
Publication:	Unpublished
Dates of experimental work:	February 11, 2005 - March 14, 2005
Guidelines:	OECD 221 "Lemna sp. Growth Inhibition Test" Revised Proposal for a New Guideline (April 2004)
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 *Lemna gibba* G3 expressed as NOEC, LOEC and EC_x for growth rate of both response variables, frond number and total frond area of plants. 3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to the nominal concentrations of 0.09, 0.30, 0.95, 3.05, 9.77, 31.3 and 100 mg p.m. (pure metabolite)/L in comparison to control. Quantitative amounts of BYI 08330-enol were measured in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

The most sensitive response variable was total frond area of plants resulting in an overall E_rC_{50} of 19.3 mg p.m./L in this study. The lowest NOEC (0.95 mg p.m./L) was based on visual effects.

MATERIAL AND METHODS

A Materials

1. Test material **BYI 08330-enol**

Description	**Beige powder**
Lot/batch No.	**Batch no.: 692-101-09-0005** **Tox no.: TOX 06850-00**
Purity	**99.1%**
Stability of test compound	**Approved until 2005-03-17**

2. Vehicle and/or positive control **No**

3. Test animals

Species	***Lemna gibba*, strain G3**
Age	**7-10 days old colonies**
Source	**Dr. [redacted], Horticulture Crops Quality Laboratory, U.S. Department of Agriculture, Beltsville, MD 20705, U.S.A.** **The plant material was transferred to the laboratory on June 27, 2002 and kept since then.**

Acclimation period	**Stock cultures are maintained in glass dishes filled with growth medium under illumination of 6.50 - 10.0 klux and temperature of 24 ± 2 °C for a minimum of three weeks. Transfers are made regularly into fresh medium to provide 7-10 days old colonies as test inoculum. All operations were conducted under sterile conditions to sustain an axenic algae culture.**
Environmental conditions	
Temperature	**24 ± 2°C**
Photoperiod	**Permanent light; nominally 6.50-10.0 klux (± 15% variation from the mean).**

B Study design and methods

1. In life dates **February 11, 2005 - February 18, 2005**

2. Experimental treatments

Test vessels consisted of glass dishes with a diameter of 10 cm and a height of 6 cm (total volume of approx. 470 mL). They were covered with lids of glass to permit gas exchange and illumination under sterile conditions. All dishes were placed in a growth incubator. In total, 3 replicate vessels per test level and 3 replicate vessels per control level were established.

The growth medium (20X-AAP) was freshly prepared according to the mentioned test.

The stock solution was prepared immediately prior to the test using 202.0 mg BYI 08330-enol and 2000 mL test medium. It was well agitated on a magnetic stirrer for 30 minutes and treated in ultrasonic bath for 10 minutes before further use. An adequate amount of the stock solution was transferred to a dilution series to obtain the concentration levels used in the study.

The range of test concentrations was selected based on pre experiments in order to define the NOE_rC, LOE_rC and E_rCx (to cover preferably the range up to 75% growth rate inhibition).

3. Observations

Observations of changes in plant development were made on study days 3, 5, and 7 (e.g. frond size, appearance, necrosis, chlorosis or gibbosity, colony break-up or loss of buoyancy, root length, morphology or breakdown).

Temperature data was recorded hourly by a data logger, pH was measured in all freshly prepared and all aged test levels and the controls, light was measured at least once. Counting of fronds and determination of total frond area were carried out using the LemnaTec Scanalyzer machine, validated for such measurements.

Test item samples were analysed for the actual concentration of BYI 08330-enol present in all freshly prepared test levels on day 0 and in all aged test levels on day 7 of the exposure period.

ECx values and confidence intervals were calculated for the stated exposure period, the LOEC determinations from the appropriate parameter (inhibition) were done using the ANOVA procedure (α = 0.05, one sided) and properly selected multiple t-tests. All statistical evaluations were done using the commercial program ToxRat Professional.

RESULTS AND DISCUSSION

A. Findings

Test conditions met all validity criteria, as the frond number increased in the control by a factor of 13.9 within 7 days corresponding to a doubling time (Td) of about 1.9 days, respectively.

The pH values ranged from 7.4 to 8.8 in the control and the incubation temperature ranged from 22.3°C to 24.0°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 8.95 klux.

The analytical findings of BYI 08330-enol found in all freshly prepared test levels on day 0 in reference to nominal concentrations ranged between 114 and 120% (average 118%). In aged test levels on days 7 there were analytical findings between 97 and 120% (average 117%) of nominal. All reported results are based on nominal initial values of the pure metabolite.

The static 7 day growth inhibition test provided the following tabulated effects:

Nominal test levels [mg p.m./L]	Final frond no. (replicate means, day 7)	Total frond area of plants (replicate means) [mm²]	% inhibition*	
			average growth rate for frond no.	average growth rate for total frond area of plants
control	167	1390	-	-
0.09	169	1457	- 0.9	- 2.9
0.30	177	1489	- 2.7	- 2.4
0.95	164	1349	0.1	0.1
3.05	133	1030	8.3	9.5
9.77	61	365	38.5	55.2
31.3	45	320	50.3	60.8
100	50	260	45.8	67.0

* negative value means growth stimulation

B. Observations

Observed visual effects:

Test level [mg p.m./L]	Observations	Effect threshold
control 0.09 0.30 0.95	no visual effects observed	NOEC
3.05	slight chlorosis on day 3 and 5 medium chlorosis on day 7	LOEC
9.77 31.3 100	medium chlorosis on day 3 strong chlorosis on days 5 and 7	

Results are based on nominal concentrations of the test item:

Endpoint (0-7 day)	Effect on frond no. [mg p.m./L]	Effect on total frond area of plants [mg p.m./L]
E_rC_{50} (CI 95%)	60.8 (21.6 – 1642)	19.3 (6.94 – 74.2)
LOE_rC	9.77	9.77
NOE_rC	3.05	3.05

CONCLUSION

The most sensitive response variable was total frond area of plants resulting in an overall E_rC_{50} of 19.3 mg p.m./L in this study. The lowest NOEC (0.95 mg p.m./L) was based on visual effects.

IIA 8.7 Effects on bees

IIA 8.7.1 Acute oral toxicity

Report:	**KIIA 8.7.1/01, [redacted]; 2004**
Title:	Effects of BYI 08330 (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory Date: 2004-07-12
source:	[redacted], Germany [redacted], Germany
Report No.:	20091035; M-081227-01-2
Publication:	Unpublished
Dates of experimental work:	June 07, 2004 – June 10, 2004
Guidelines:	OECD 213: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Oral Toxicity Test, (adopted 21st September 1998) OECD 214: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Contact Toxicity Test, (adopted 21st September 1998) recent recommendations of the ICPBR group, held in Avignon, France, 1999
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine acute oral and contact toxicity of BYI 08330 tech. to Honey bees (*Apis mellifera* L.) in a limit test. 5 replicates, each consisting of 10 bees in one cage per concentration were exposed to test concentrations of 100 µg a.s./bee in the contact test and 107.3 µg a.s./bee in the oral test. Mortality was assessed after 4, 24 and 48 hours. Dimethoate 400 g/L (nominal) was used as toxic reference.
No test item induced behavioural effects were observed at any time. Since mortality levels in the 100.0 µg a.s./bee group are below 2% the contact LD_{50} (48 h) can be considered as > 100.0 µg a.s./bee. Since mortality levels in the 107.3 µg a.s./bee group are below 4% the oral LD_{50} (48 h) can be considered as > 107.3 µg a.s./bee.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**Solid, white**
Lot/batch No.	**Batch no.: 08045/0014 (mixed batch)** **Tox no.: 06695-00**
Purity	**97.2% (w/w)**
Stability of test compound	**Expiry date: 2004-11-04, when stored at room temperature**
2. Vehicle and/or positive control	**Acetone** **Perfekthion EC 400 (analysed content: Dimethoate: 396.1 g/L)**

3. Test animals

Species	Honey bee (*Apis mellifera* L.), working bees
Age	approx. 4-6 weeks
Source	Honey bee colonies, disease-free and queen-right, bred by IBACON
Collection	Collected in the morning of use.
Environmental conditions	Incubators
Temperature	25°C
Relative humidity	58 - 72%
Photoperiod	24 h darkness (except during observation)
Ventilation	Ventilation to avoid possible accumulation of pesticide vapour

B Study design and methods

1. In life dates June 07, 2004 – June 10, 2004

2. Experimental treatments

Test units were stainless steel cages of 10 cm x 8.5 cm x 5.5 cm (length x width x height), the front side was a removable glass sheet, the bottom was perforated with 98 ventilation holes (Ø 1 mm), the inner walls were lined with filter paper. 10 bees were used per test unit, 5 replicates per test item dose level, controls and toxic standard dosages (i.e. 50 individuals per treatment group). Food was commercial ready-to-use syrup (Apiinvert; 30% Saccharose, 31% Glucose, 39% Fructose). Nominal dosage of the test item in the contact and in the oral test was 100 µg a.s./bee. Nominal dosage of the toxic reference was 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee (contact test) and 0.30, 0.15, 0.08 and 0.04 µg dimethoate/bee (oral test). In the contact test a CO_2/tap water + Adhaesit and a CO_2/acetone treated control were run in parallel; in the oral test a tap water/sugar and an acetone/sugar control.

<u>Application of the test item in the contact test:</u>

Bees were anaesthetized with CO_2 in the contact test. A single 5 µL droplet of BYI 08330 (100 µg a.s./bee) in solvent (solvent = acetone) was placed on the ventral bee thorax using a Burkard - Applicator. For the controls one 5 µL droplet of a) acetone and b) tap water with 1% Adhaesit was used. The toxic standard was applied in 5 µL Acetone (a 5 µL droplet was chosen in deviation to the guideline recommendation of 1 µL, since a higher volume ensured a more reliable dispersion of the test item. IBACON experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected).

<u>Application of the test item in the oral test:</u>

Food (syrup) was mixed with BYI 08330, toxic standard and tap water/acetone (1 part solvent solution +19 parts syrup). This diet was offered to the bees after 20 min. starvation time in syringes which were weighed before (after 6 hrs the test item treated food was replaced by fresh, untreated food).

3. Observations

The number of dead bees was assessed after 4 hours (first day); 24 and 48 hours. Behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4 hours (first day); 24 and 48 hours.
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 toxic standard was estimated with Moving Average Computations (after [redacted] 1947). The LD_{50} calculation was carried out taking into account the mortality data corrected by control mortality using Abbott's formula (1925). The software used to perform the statistical analysis was ToxRat® Professional, Version 2.07.

RESULTS AND DISCUSSION

A. Findings

The validity criteria were met as mortality in the water and solvent (acetone) control groups were ≤ 10% and the LD_{50} 24 h values for the toxic standard were in the postulated range of 0.1 - 0.30 µg a.s./bee (contact) and 0.1 - 0.35 µg a.s./bee (oral), see also tables in the following.

Mortality and behavioural abnormalities of the bees in the contact toxicity test (results are average from 5 replicates (ten bees each) per dosage/control)

	after 4 hours		after 24 hours		after 48 hours	
Dosage [µg a.s./bee]	mortality mean [%]	behav. abnorm. mean [%]	mortality mean [%]	behav. abnorm. mean [%]	mortality mean [%]	behav. abnorm. mean [%]
Test item						
100	0.0	0.0	0.0	0.0	2.0	0.0
Water	0.0	0.0	0.0	0.0	2.0	0.0
Solvent	0.0	0.0	0.0	0.0	2.0	0.0
Toxic standard						
0.30	20.0	34.0	94.0	4.0	96.0	0.0
0.20	28.0	12.0	70.0	2.0	82.0	0.0
0.15	18.0	6.0	46.0	8.0	62.0	0.0
0.10	0.0	0.0	0.0	0.0	2.0	0.0

behav. abnorm. = behavioural abnormalities
water = CO_2/water treated control, solvent = CO_2/solvent treated control

In the oral toxicity test the maximum nominal test level of spirotetramat (100 µg a.s./bee) corresponded to an actual intake of 107.3 µg a.s./bee. This concentration level led to 4.0% mortality after 48 hours.

Mortality and behavioural abnormalities of the bees in the oral toxicity test (results are average from 5 replicates (ten bees each) per dosage/control)

	after 4 hours		after 24 hours		after 48 hours	
Dosage [µg a.s./bee]	mortality mean [%]	behav. abnorm. mean [%]	mortality mean [%]	behav. abnorm. mean [%]	mortality mean [%]	behav. abnorm. mean [%]
Test item						
107.3	0.0	0.0	2.0	0.0	4.0	0.0
Water	0.0	0.0	0.0	2.0	2.0	0.0
Solvent	0.0	0.0	0.0	0.0	0.0	0.0
Toxic standard						
0.24	28.0	24.0	94.0	0.0	94.0	0.0
0.12	0.0	0.0	22.0	0.0	26.0	0.0
0.07	0.0	0.0	14.0	0.0	22.0	0.0
0.04	0.0	0.0	2.0	0.0	2.0	0.0

behav. abnorm. = behavioural abnormalities
water = CO_2/water treated control, solvent = CO_2/solvent treated control

Oral and contact toxicity LD_{50} values of bees treated with BYI 08330

	LD_{50} [µg a.s./bee]	
	Contact test	Oral test
Test item (48 h)	**> 100.0**	**> 107.3**
Toxic standard (24 h)	**0.17**	**0.15**
(95% Confidence limit)	**(0.15 - 0.18)**	**(0.13 - 0.16)**

B. Observations

No behavioural abnormalities attributed to exposure of the test item to the bees occurred during the experimental time of 48 hours.

CONCLUSION

Toxicity of BYI 08330 was tested in both an acute contact and an oral toxicity test on honey bees. The LD_{50} (48 h) was > 100 µg a.s./bee in the contact toxicity test, the LD_{50} (48 h) was > 107.3 µg a.s./bee in the oral toxicity test.

Report:	**KIIA 8.7.1/02, [redacted], 2010a**
Title:	Effects of spirotetramat-cis-enol (Acute Contact and Oral) on Honey Bees (*Apis mellifera L.*) in the Laboratory Date: 2010-11-24
Organisation	[redacted], Germany
Report No:	58821035; M-395773-01-1
Publication:	unpublished
Dates of experimental work:	July 6 to July 23, 2010
Guidelines:	OECD 213 and 214 (1998), Recent recommendations of the ICPBR group, held in Avignon, France, 1999
Deviations:	No major deviations
GLP:	Yes

Executive summary

The aim of the study was to determine acute oral and contact toxicity of spirotetramat-cis-enol (AE 1302944, metabolite of spirotetramat) to honey bees (*Apis mellifera* L.) in a limit test under laboratory conditions.

Five replicates, each consisting of 10 worker bees in one cage per concentration were exposed to test concentrations of 100 µg a.s./bee in the contact test and 110.7 µg a.s./bee (based on the actual intake of the test item in the oral test for 48 hours.

Mortality and behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4, 24 and 48 hours. No remarked reactions to exposure of the test item were noted in any of the test bees throughout the entire study (contact & oral test).

The contact LD_{50} (48 h) can be considered as > 100.0 µg a.s./bee.

The oral LD_{50} (48 h) can be considered as > 110.7 µg a.s./bee.

MATERIAL AND METHODS

A Materials

1. Test material	Spirotetramat-cis-enol (BYI-08330-cis-enol); metabolite of spirotetramat
Description	Solid, beige
Lot/batch No.	Batch Code: AE 1302944 00 1B99 0002 Tox.No.: TOX 09049-00
Purity	99.1% (w/w) analytical
Stability of test compound	Expiry date: 2010-11-12, when stored at +20 °C ± 5°C, in the dark
2. Positive control	Oral Test: a) 50% w/w aqueous sugar solution (with tap water) b) 50% w/w sugar solution with solvent (45% water, 5% acetone, 50% sugar) Contact Test: a) Tap water with 0.5% Adhäsit[1] (applied after anesthetization with CO2) b) Acetone (applied after anesthetization with CO2)
Negative Control (Reference Item)	Perfekthion, 400 g dimethoate/L nominal 414.8 g/L analysed

[1] Adhäsit improves spreading of the test droplet on the water-repellent hairs on the thorax of bees

3. Test animals	
Species	Worker honey bees (*Apis mellifera carnica* L.)
Age/sex	Adult, female
Source	Honey bee colonies, disease-free and queen-right, bred by IBACON
Collection	With glass tubes, from the flight board without the use of smoke and without anaesthetics, collected on the morning of use
Environmental conditions	Incubators
Temperature	24 - 25°C
Relative humidity	65 - 93%
Photoperiod	24 h darkness (except during observation)
Ventilation	Ventilation to avoid possible accumulation of pesticide vapour

B Study design and methods

1. In life dates: July 06 - July 23, 2010

2. Experimental treatments

Test units were stainless steel cages of 10 cm x 8.5 cm x 5.5 cm (length x width x height), the front side was a removable glass sheet, the bottom was perforated with 98 ventilation holes (Ø 1 mm), the inner walls were lined with filter paper. 10 bees were used per test unit, 5 replicates per test item dose level, controls and toxic standard dosages (i.e. 50 individuals per treatment group). Food was commercial ready-to-use syrup (Apiinvert; 30% Sucrose, 31% Glucose, 39% Fructose).
Nominal dosage of the test item in the contact and in the oral test was 100 µg a.s./bee. Nominal dosage of the toxic reference was 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee (contact test) and 0.30, 0.15, 0.08 and 0.04 µg dimethoate/bee (oral test). In the contact test a CO_2/tap water + Adhaesit and a CO_2/acetone treated control were run in parallel; in the oral test a tap water/sugar and an acetone/sugar control.

Application of the test item in the contact test:

Bees were anaesthetized with CO_2 in the contact test. A single 5 µL droplet of BYI 08330-cis-enol (100 µg a.s./bee) in an appropriate carrier (acetone) was placed on the dorsal bee thorax using a Burkard - Applicator. For the controls, one 5 µL droplet of a) tap water containing 0.5 % Adhäsit and b) pure acetone was used. The reference item was also applied in a 5 µL droplet (dimethoate made up in acetone).
A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item; IBACON experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected; [presented as a poster on the ICPBR Bee Protection Group meeting in Bologna, 2002].

Application of the test item in the oral test:

Appropriate amounts of BYI 08330-cis-enol or reference item dilutions in acetone were mixed with syrup (ready-to-use syrup; Apiinvert) in order to achieve the required test concentrations in a final dilution of 50 % syrup solution (45% water, 50% syrup and 5% acetone (w/w)). For the controls, the same proportion of syrup, water and acetone was used (solvent control) and similarly, 50 % aqueous syrup solution was used for the negative control.

The treated food was offered to the bees after 20 min starvation time in syringes which were weighed before and after introduction into the cages. After 3 hrs 55 min (when

uptake was completed) the test item treated food was replaced by fresh, untreated food. The mean target dose levels (*e.g.* 100 µg a.s./bee nominal) would have been obtained if exactly 20 mg/bee of the treated food were ingested. In practice, uptake of the treated sugar solutions differed slightly from the nominal 20 mg/bee and results are given based on the measured consumption (= 110.7 µg a.s./bee measured dose level).

3. Observations

The number of dead bees was assessed after 4 hours (first day); 24 and 48 hours. Behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4 hours (first day); 24 and 48 hours.

Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the control in both the contact and oral tests.

The contact and oral LD_{50} values of the reference item were estimated using the binomial distribution (according to [redacted] 1977).

The LD_{50} calculation was carried out taking into account the mortality data corrected by control (solvent control) mortality using Abbott's formula (1925).

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

RESULTS AND DISCUSSION

A. Findings

Validity of the test

The contact and oral test are considered valid as the control mortality in each case was < 10% and the LD_{50} values obtained with the reference item (dimethoate), were within the required ranges (see also tables below).

Contact Toxicity Test:

Mortality

At the end of the contact toxicity test (48 hours after application), there was 2.0% mortality at 100.0 µg a.s./bee. Also 2.0% mortality occurred in the water control group (water + 0.5 % Adhäsit) and in the solvent control group (acetone), respectively.

Behavioural abnormalities

No remarked reactions to exposure of the test item were noted in any of the test bees throughout the duration of the study.

Since 2.0% mortality occurred in the 100.0 µg a.s./bee group, the contact LD_{50} can be considered as > 100.0 µg a.s./bee.

Mortality and behavioural abnormalities of the bees in the contact toxicity test

	after 4 hours		after 24 hours		after 48 hours	
	mortality	behav. abnorm.	mortality	behav. abnorm.	mortality	behav. abnorm.
dosage	mean	mean	mean	mean	mean	mean
	%	%	%	%	%	%
test item µg a.i./bee						
100.0	0.0	0.0	2.0	0.0	2.0	0.0
water	0.0	0.0	0.0	0.0	2.0	0.0
solvent	0.0	0.0	0.0	0.0	2.0	0.0
reference item µg a.i./bee						
0.30	0.0	6.0	82.0	8.0	92.0	0.0
0.20	0.0	4.0	42.0	34.0	66.0	10.0
0.15	0.0	0.0	10.0	30.0	26.0	4.0
0.10	0.0	0.0	0.0	0.0	2.0	0.0

results are averages from five replicates (ten bees each) per dosage/control
see Appendix 1, Table 4 for details; behav. abnorm. = behavioural abnormalities;
water = CO_2/water-treated control; solvent = CO_2/solvent control

Oral Toxicity Test:

Mortality

In the oral toxicity test the maximum nominal test level of spirotetramat-cis-enol (100 µg a.s./bee) corresponded to an actual intake of 110.7 µg a.s./bee. This concentration level led to no mortality after 48 hours.

Behavioural abnormalities

No remarked reactions to exposure of the test item were noted in any of the test bees throughout the duration of the study.

There was no mortality in the 110.7 µg a.s./bee group, therefore the oral LD_{50} can be considered as > 110.7 µg a.s./bee.

Mortality and behavioural abnormalities of the bees in the oral toxicity test

consumed dosage	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 µg a.i./bee						
110.7	0.0	0.0	0.0	0.0	0.0	0.0
water	0.0	0.0	0.0	0.0	2.0	0.0
solvent	0.0	0.0	0.0	0.0	0.0	0.0
reference item µg a.i./bee						
0.30	36.0	32.0	100.0	0.0	100.0	0.0
0.15	0.0	0.0	66.0	6.0	72.0	0.0
0.08	0.0	0.0	6.0	2.0	8.0	0.0
0.05	0.0	0.0	8.0	0.0	8.0	0.0

results are averages from five replicates (ten bees each) per dosage/control
see Appendix 1, Table 6 for details; behav. abnorm. = behavioural abnormalities
water = water control; solvent = solvent control

Oral and contact toxicity LD_{50} values of bees treated with BYI 08330-cis-enol:

	LD_{50} [µg a.s./bee]	
	Contact test	Oral test
Test item (48 h)	> 100.0	> 110.7
Toxic standard (24 h)	0.22	0.13
(95% Confidence limit)	(0.15 - 0.30)	(0.08 - 0.15)

B. Observations

No behavioural abnormalities attributed to exposure of the test item to the bees occurred during the experimental time of 48 hours.

CONCLUSION

The toxicity of spirotetramat-cis-enol was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD_{50} (48 h) was > 100.0 µg a.s./bee.
The oral LD_{50} (48 h) was > 110.7 µg a.s./bee.

Report:	**KIIA 8.7.1/03, [redacted], 2010b**
Title:	Effects of spirotetramat-cis-ketohydroxy (Acute Contact and Oral) on Honey Bees (*Apis mellifera L.*) in the Laboratory Date: 2010-11-24
Organisation:	[redacted], Germany
Report No:	58831035; M-395781-01-1
Publication:	unpublished
Dates of experimental work:	May 4 to May 7, 2010
Guidelines:	OECD 213 and 214 (1998)
Deviations:	No major deviations
GLP:	Yes

Executive summary

The aim of the study was to determine acute oral and contact toxicity of spirotetramat-cis-ketohydroxy (AE 1422479, metabolite of spirotetramat) to honey bees (*Apis mellifera* L.) in a limit test under laboratory conditions.

Five replicates, each consisting of 10 worker bees in one cage per concentration were exposed to test concentrations of 100 µg a.s./bee in the contact test and 105.5 µg a.s./bee (based on the actual intake of the test item) in the oral test for 48 hours.

Mortality and behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4, 24 and 48 hours. No remarked reactions to exposure of the test item were noted in any of the test bees throughout the entire study (contact & oral test).

The contact LD_{50} (48 h) can be considered as > 100.0 µg a.s./bee.

The oral LD_{50} (48 h) can be considered as > 105.5 µg a.s./bee.

Material and Methods

A Materials

1. Test material

Description	Spirotetramat-cis-ketohydroxy (BYI-08330-cis-keto-hydroxy), metabolite of spirotetramat
Lot/batch No.	Solid, light yellow Batch Code.: AE 1422479-01-01 (original batch: NLL 7549-7) Tox No.: TOX 08972-00
Purity	91.4 % (w/w)
Stability of test compound	Expiry date: 2010-09-17, when stored at +20 °C ± 5°C

2. Positive control

Oral Test:
a) 50% w/w aqueous sugar solution (with tap water)
b) 50% w/w sugar solution with solvent (45% water, 5% acetone, 50% sugar)
Contact Test:
a) Tap water with 0.5% Adhäsit[2] (applied after anesthetization with CO2)

[2] Adhäsit improves spreading of the test droplet on the water-repellent hairs on the thorax of bees

	b) Acetone (applied after anesthetization with CO2)
Negative Control (Reference Item)	Perfekthion, 400 g dimethoate/L nominal 414.8 g/L analysed
3. Test animals	
Species	Worker honey bees (*Apis mellifera carnica L.)*
Age/sex	Adult, female
Source	Honey bee colonies, disease-free and queen-right, bred by IBACON
Collection	With glass tubes, from the flight board without the use of smoke and without anaesthetics, collected on the morning of use
Environmental conditions	Incubators
Temperature	25°C
Relative humidity	50 - 70%
Photoperiod	24 h darkness (except during observation)
Ventilation	Ventilation to avoid possible accumulation of pesticide vapour

B Study design and methods

1. In life dates: May 04 – May 07, 2010

2. Experimental treatments

Test units were stainless steel cages of 10 cm x 8.5 cm x 5.5 cm (length x width x height), the front side was a removable glass sheet, the bottom was perforated with 98 ventilation holes (Ø 1 mm), the inner walls were lined with filter paper. 10 bees were used per test unit, 5 replicates per test item dose level, controls and toxic standard dosages (i.e. 50 individuals per treatment group). Food was commercial ready-to-use syrup (Apiinvert; 30% Sucrose, 31% Glucose, 39% Fructose).
Nominal dosage of the test item in the contact and in the oral test was 100 µg a.s./bee. Nominal dosage of the toxic reference was 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee for the contact test. Measured dosage was 0.26, 0.16, 0.08 and 0.05 µg dimethoate/bee for the oral test. In the contact test a CO_2/tap water + Adhaesit and a CO_2/acetone treated control were run in parallel; in the oral test a tap water/sugar and an acetone/sugar control.

Application of the test item in the contact test:

Bees were anaesthetized with CO_2 in the contact test. A single 5 µL droplet of BYI 08330-cis-ketohydroxy (100 µg a.s./bee) in an appropriate carrier (acetone) was placed on the dorsal bee thorax using a Burkard - Applicator. For the controls, one 5 µL droplet of a) tap water containing 0.5% Adhäsit and b) pure acetone was used. The reference item was also applied in a 5 µL droplet (dimethoate made up in acetone).

A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item; IBACON experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected; [presented as a poster on the ICPBR Bee Protection Group meeting in Bologna, 2002].

Application of the test item in the oral test:

Appropriate amounts of BYI 08330-cis-ketohydroxy or reference item dilutions in acetone were mixed with syrup (ready-to-use syrup; Apiinvert) in order to achieve the required test concentrations in a final dilution of 50% syrup solution (45% water, 50%

syrup and 5% acetone (w/w)). For the controls, the same proportion of syrup, water and acetone was used (solvent control) and similarly, 50% aqueous syrup solution was used for the negative control.

The treated food was offered to the bees after 20 min starvation time in syringes which were weighed before and after introduction into the cages. After 2 hrs 45 min (when uptake was completed) the test item treated food was replaced by fresh, untreated food. The mean target dose levels (*e.g.* 100 µg a.s./bee nominal) would have been obtained if exactly 20 mg/bee of the treated food were ingested. In practice, uptake of the treated sugar solutions differed slightly from the nominal 20 mg/bee and results are given based on the measured consumption (= 105.5 µg a.s./bee measured dose level).

3. Observations

The number of dead bees was assessed after 4 hours (first day); 24 and 48 hours. Behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4 hours (first day); 24 and 48 hours.

Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the control in both the contact and oral tests.

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

If necessary, the LD_{50} calculation was carried out taking into account the mortality data corrected by control (solvent control) mortality using Abbott's formula (1925).

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

RESULTS AND DISCUSSION

A. Findings

Validity of the test

The contact and oral test are considered valid as the control mortality in each case was < 10% and the LD_{50} values obtained with the reference item (dimethoate) were within the required ranges (see also tables below).

Deviations to the study plan

The test item was stored at +20°C ± 5°C with a minimum temperature of 19°C for up to 20 days instead of +25°C ± 5°C as indicated in the study plan.

Because of technical (climatic) reasons the temperature had a broader range than expected.

No negative impact on the study is to be expected from this minor deviation as - according to the certificate of analysis - deviate storage conditions for several days are acceptable.

Contact Toxicity Test:

Mortality

At the end of the contact toxicity test (48 hours after application), there was 2.0% mortality at 100.0 µg a.s./bee. Also 2.0% mortality occurred in the water control

group (water + 0.5 % Adhäsit) and in the solvent control group (acetone), respectively.

Behavioural abnormalities
One bee out of the 50 bees in the test item treatment showed a discoordinated movement.

Since 2.0% mortality occurred in the 100.0 µg a.s./bee group, the contact LD_{50} can be considered as > 100.0 µg a.s./bee.

Mortality and behavioural abnormalities of the bees in the contact toxicity test

	after 4 hours		after 24 hours		after 48 hours	
	mortality	behav. abnorm.	mortality	behav. abnorm.	mortality	behav. abnorm.
dosage	mean %	mean %	mean %	mean %	mean %	mean %
test item µg a.i./bee						
100.0	0.0	0.0	0.0	2.0	2.0	0.0
water	2.0	0.0	2.0	0.0	2.0	0.0
solvent	0.0	0.0	2.0	0.0	2.0	0.0
reference item µg a.i./bee						
0.30	0.0	24.0	98.0	2.0	100.0	0.0
0.20	0.0	12.0	78.0	8.0	90.0	2.0
0.15	0.0	0.0	46.0	0.0	64.0	6.0
0.10	0.0	0.0	0.0	0.0	0.0	0.0

results are averages from five replicates (ten bees each) per dosage/control
see Appendix 1, Table 4 for details; behav. abnorm. = behavioural abnormalities;
water = CO_2/water-treated control; solvent = CO_2/solvent control

Oral Toxicity Test:

Mortality
In the oral toxicity test the maximum nominal test level of spirotetramat-cis-ketohydroxy (100 µg a.s./bee) corresponded to an actual intake of 105.5 µg a.s./bee. This concentration level led to no mortality after 48 hours. In the solvent (acetone) and water control (50% sugar solution) also no mortality occurred, respectively.

Behavioural abnormalities
No test item related behavioural abnormalities occurred.

There was no mortality in the 105.5 µg a.s./bee group, therefore the oral LD_{50} can be considered as > 105.5 µg a.s./bee.

Mortality and behavioural abnormalities of the bees in the oral toxicity test

consumed dosage	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 µg a.i./bee						
105.5	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
solvent	0.0	0.0	0.0	0.0	0.0	0.0
reference item µg a.i./bee						
0.26	50.0	28.0	98.0	2.0	100.0	0.0
0.16	18.0	22.0	96.0	2.0	96.0	0.0
0.08	6.0	4.0	16.0	0.0	18.0	0.0
0.05	0.0	0.0	0.0	0.0	0.0	0.0

results are averages from five replicates (ten bees each) per dosage/control
see Appendix 1, Table 6 for details; behav. abnorm. = behavioural abnormalities
water = water control; solvent = solvent control

Oral and contact toxicity LD_{50} values of bees treated with BYI 08330-cis-ketohydroxy:

	LD_{50} [µg a.s./bee] Contact test	Oral test
Test item (48 h)	> 100.0	> 105.5
Toxic standard (24 h)	0.16	0.11
(95% Confidence limit)	(0.14 - 0.18)	(0.10 - 0.12)

B. Observations

Contact test: One bee out of the 50 bees in the test item treatment showed uncoordinated movements.

Oral test: No behavioural abnormalities attributed to exposure of the test item to the bees occurred during the experimental time of 48 hours in the oral test.

CONCLUSION

The toxicity of spirotetramat-cis-ketohydroxy was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD_{50} (48 h) was > 100.0 µg a.s./bee.
The oral LD_{50} (48 h) was > 105.5 µg a.s./bee.

Report:	**KIIA 8.7.1/04, [redacted], 2010c**
Title:	Effects of spirotetramat-enol-glucoside (Acute Contact and Oral) on Honey Bees (*Apis mellifera L.*) in the Laboratory Date: 2010-11-25
Organisation:	[redacted], Germany
Report No:	58851035; M-395843-01-1
Publication:	unpublished
Dates of experimental work:	May 25 to May 28, 2010
Guidelines:	OECD 213 and 214 (1998)
Deviations:	No major deviations
GLP:	Yes

Executive summary

The aim of the study was to determine acute oral and contact toxicity of spirotetramat—enol-glucoside (AE 1935398, metabolite of spirotetramat) to honey bees (*Apis mellifera* L.) in a limit test under laboratory conditions.

Five replicates, each consisting of 10 worker bees in one cage per concentration were exposed to test concentrations of 100 µg a.s./bee in the contact test and 109.1 µg a.s./bee (based on the actual intake of the test item in the oral test for 48 hours.

Mortality and behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4, 24 and 48 hours. No remarked reactions to exposure of the test item were noted in any of the test bees throughout the entire study (contact & oral test).

The contact LD_{50} (48 h) can be considered as > 100.0 µg a.s./bee.

The oral LD_{50} (48 h) can be considered as > 109.1 µg a.s./bee.

Material and Methods

A Materials

1. Test material	Spirotetramat-enol-glucoside (BYI-08330-enol-glucoside); metabolite of spirotetramat
Description	light beige powder
Lot/batch No.	Batch Code:: AE 1935398-01-01, original batch: 2006BRP027-209
Purity	90.9% (w/w) analytical
Stability of test compound	Expiry date: 2010-11-02, when stored at - 20 °C ± 5°C, in the dark, flush with nitrogen and seal tightly after use (hygroscopic substance)
2. Positive control	Oral Test: a) 50% w/w aqueous sugar solution (with tap water) b) 50% w/w sugar solution with solvent (45% water, 5% acetone, 50% sugar) Contact Test: a) Tap water with 0.5% Adhäsit[3] (applied after anesthetization with CO2) b) Acetone (applied after anesthetization with CO2)

[3] Adhäsit improves spreading of the test droplet on the water-repellent hairs on the thorax of bees

Negative Control (Reference Item)	Perfekthion, 400 g dimethoate/L nominal 414.8 g/L analysed
3. Test animals	
Species	Worker honey bees (*Apis mellifera carnica* L.)
Age/sex	Adult, female
Source	Honey bee colonies, disease-free and queen-right, bred by IBACON
Collection	With glass tubes, from the flight board without the use of smoke and without anaesthetics, collected on the morning of use
Environmental conditions	Incubators
Temperature	24 - 25°C
Relative humidity	54 - 90%
Photoperiod	24 h darkness (except during observation)
Ventilation	Ventilation to avoid possible accumulation of pesticide vapour

B Study design and methods

1. In life dates: July 06 - July 23, 2010

2. Experimental treatments

Test units were stainless steel cages of 10 cm x 8.5 cm x 5.5 cm (length x width x height), the front side was a removable glass sheet, the bottom was perforated with 98 ventilation holes (Ø 1 mm), the inner walls were lined with filter paper. 10 bees were used per test unit, 5 replicates per test item dose level, controls and toxic standard dosages (i.e. 50 individuals per treatment group). Food was commercial ready-to use syrup (Apiinvert; 30% Sucrose, 31% Glucose, 39% Fructose).
Nominal dosage of the test item in the contact and in the oral test was 100 µg a.s./bee.
Nominal dosage of the toxic reference was 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee (contact test) and 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee (oral test). Measured dose level of the toxic reference in the oral test was 0.32, 0.16, 0.08 and 0.06 µg dimethoate/bee.
In the contact test a CO_2/tap water + Adhaesit and a CO_2/acetone treated control were run in parallel; in the oral test a tap water/sugar and an acetone/sugar control.

Application of the test item in the contact test:

Bees were anaesthetized with CO_2 in the contact test. A single 5 µL droplet of BYI 08330-enol-glucoside (100 µg a.s./bee) in an appropriate carrier (acetone) was placed on the dorsal bee thorax using a Burkard - Applicator. For the controls, one 5 µL droplet of a) tap water containing 0.5% Adhäsit and b) pure acetone was used. The reference item was also applied in a 5 µL droplet (dimethoate made up in acetone).

A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item; IBACON experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected; [presented as a poster on the ICPBR Bee Protection Group meeting in Bologna, 2002].

Application of the test item in the oral test:

Appropriate amounts of BYI 08330-enol-glucoside or reference item dilutions in acetone were mixed with syrup (ready-to-use syrup; Apiinvert) in order to achieve the required test concentrations in a final dilution of 50% syrup solution (45% water, 50% syrup and 5% acetone (w/w)). For the controls, the same proportion of syrup, water and acetone

was used (solvent control) and similarly, 50% aqueous syrup solution was used for the negative control.

The treated food was offered to the bees after 20 min starvation time in syringes which were weighed before and after introduction into the cages. After 50 minutes (when uptake was completed) the test item treated food was replaced by fresh, untreated food. The mean target dose levels (*e.g.* 100 µg a.s./bee nominal) would have been obtained if exactly 20 mg/bee of the treated food were ingested. In practice, uptake of the treated sugar solutions differed slightly from the nominal 20 mg/bee and results are given based on the measured consumption (= 109.1 µg a.s./bee measured dose level).

3. Observations

The number of dead bees was assessed after 4 hours (first day), 24 and 48 hours. Behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4 hours (first day); 24 and 48 hours.

Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the control in both the contact and oral tests.

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

The LD_{50} calculation was carried out taking into account the mortality data corrected by control (solvent control) mortality using Abbott's formula (1925).

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Results and Discussion

A. Findings

Validity of the test

The contact and oral test are considered valid as the control mortality in each case was < 10% and the LD_{50} values obtained with the reference item (dimethoate), were within the required ranges (see also tables below).

Contact Toxicity Test:

Mortality

At the end of the contact toxicity test (48 hours after application), there was no mortality at 100.0 µg a.s./bee. Also no mortality occurred in the water control group (water + 0,5 % Adhäsit).

Behavioural abnormalities

No remarked reactions to exposure of the test item were noted in any of the test bees throughout the duration of the study.

Since no mortality occurred in the 100.0 µg a.s./bee group, the contact LD_{50} can be considered as > 100.0 µg a.s./bee.

Mortality and behavioural abnormalities of the bees in the contact toxicity test

	after 4 hours		after 24 hours		after 48 hours	
	mortality	behav. abnorm.	mortality	behav. abnorm.	mortality	behav. abnorm.
dose	mean %	mean %	mean %	mean %	mean %	mean %
test item µg a.i./bee						
100.0	0.0	0.0	0.0	0.0	0.0	0.0
water	0.0	0.0	0.0	0.0	0.0	0.0
reference item µg a.i./bee						
0.30	14.0	28.0	84.0	6.0	90.0	0.0
0.20	2.0	0.0	46.0	14.0	58.0	0.0
0.15	0.0	0.0	20.0	4.0	32.0	0.0
0.10	0.0	0.0	8.0	0.0	14.0	0.0

results are averages from five replicates (ten bees each) per dosage/control
see Appendix 1, Table 4 for details;
behav. abnorm. = behavioural abnormalities; water = CO_2/water-treated control

Oral Toxicity Test:

Mortality

In the oral toxicity test the maximum nominal test level of spirotetramat-enol-glucoside (100 µg a.s./bee) corresponded to an actual intake of 109.1 µg a.s./bee. This concentration level led to no mortality after 48 hours. 20% mortality occurred in the control group (50% sugar solution).

Behavioural abnormalities

No remarked reactions to exposure of the test item were noted in any of the test bees throughout the duration of the study.

There was no mortality in the 109.1 µg a.s./bee group, therefore the oral LD_{50} can be considered as > 109.1 µg a.s./bee.

Mortality and behavioural abnormalities of the bees in the oral toxicity test

consumed dosage	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 µg a.i./bee						
109.1	0.0	0.0	0.0	0.0	0.0	0.0
water	0.0	0.0	0.0	0.0	2.0	0.0
reference item µg a.i./bee						
0.32	56.0	42.0	100.0	0.0	100.0	0.0
0.16	4.0	30.0	70.0	4.0	82.0	0.0
0.08	0.0	0.0	4.0	2.0	8.0	0.0
0.06	0.0	0.0	0.0	2.0	4.0	0.0

results are averages from five replicates (ten bees each) per dosage/control
see Appendix 1, Table 6 for details; water = water/sugar treated control
behav. abnorm. = behavioural abnormalities

Oral and contact toxicity LD_{50} values of bees treated with BYI 08330-enol-glucoside:

	LD_{50} [µg a.s./bee]	
	Contact test	Oral test
Test item (48 h)	> 100.0	> 109.1
Toxic standard (24 h)	0.21	0.16
(95% Confidence limit)	(0.18 - 0.24)	(0.14 - 0.18)

B. Observations

No behavioural abnormalities attributed to exposure of the test item to the bees occurred during the experimental time of 48 hours for both the contact and the oral toxicity test.

CONCLUSION

The toxicity of spirotetramat-enol-glucoside was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD_{50} (48 h) was > 100.0 µg a.s./bee.
The oral LD_{50} (48 h) was > 109.1 µg a.s./bee.

Report:	**KIIA1 8.7.1/05, [redacted] 2011**
Title:	Effects of spirotetramat-mono-hydroxy (Acute Contact and Oral) on Honey Bees (*Apis mellifera L.*) in the Laboratory Date: 2011-08-10
Organisation:	[redacted], Germany
Report No:	58841035; M-411975-01-1
Publication:	unpublished
Dates of experimental work:	May 04 (contact test) – May 12, 2011 (2nd oral test)
Guidelines:	OECD 213 and 214 (1998)
Deviations:	No major deviations
GLP:	Yes

Executive summary

The aim of the study was to determine acute oral and contact toxicity of spirotetramat-mono-hydroxy (AE 1302944, metabolite of spirotetramat) to honey bees (*Apis mellifera* L.) in a limit test under laboratory conditions.
Under laboratory conditions *Apis mellifera*, 50 worker bees were exposed for 48 hours to a single dose of 100.0 µg a.s. per bee by topical application (contact limit test); in parallel, 30 worker bees per dose were exposed for 72 hours to doses of 71.5, 42.4, 28.1, 13.9 and 7.0 µg a.s. per bee by feeding (1st definitive oral dose response test, values based on the actual intake of the test item). Since the observations in the 1st definitive oral dose response test were not in line with the data of the range-finding test, it was decided, in order to allow for a profound judgement, to conduct a further (2nd) definitive oral toxicity test, by also increasing the number of replicates to enhance the explanatory power of the results. In this further definitive oral toxicity test with an increased number of replicates, 60 worker bees per dose were exposed for 48 hours to doses of 101.6, 55.3, 26.3, 13.6 and 6.7 µg a.s. per bee by feeding (2nd definitive oral dose response test, increased number of replicates, values based on the actual intake of the test item).

Mortality and behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4, 24 and 48 hours (72 hours in 1st oral test). No remarked reactions of the bees to exposure of the test item were noted in the two oral tests; whereas 3 bees showed uncoordinated movements in the contact test.

The contact LD_{50} (48 h) can be considered as > 100.0 µg a.s./bee
The oral LD_{50} (48 h) can be considered as > 101.6 µg a.s./bee.

MATERIAL AND METHODS

A Materials

1. Test material	Spirotetramat-mono-hydroxy (BYI-08330-mono-hydroxy); metabolite of spirotetramat
Description	Solid white
Lot/batch No.	Batch Code.: AE 1796847-PU-01, original batch: NLL 7635-2D
Purity	96.3% (w/w) analytical
Stability of test compound	Expiry date: 2010-09-16 (1st test), 2011-07-28 (2nd test) when stored at +10 °C to 30°C in the dark
2. Positive control	Oral Test: a) 50% w/w aqueous sugar solution (with tap water) b) 50% w/w sugar solution with solvent (45% water, 5% acetone, 50% sugar) Contact Test: a) Tap water with 0.5% Adhäsit[4] (applied after anesthetization with CO2) b) Acetone (applied after anesthetization with CO2)
Negative Control (Reference Item)	Perfekthion, 400 g dimethoate/L nominal 414.8 g/L analysed
3. Test animals	
Species	Worker honey bees (*Apis mellifera carnica* L.)
Age/sex	Adult, female
Source	Honey bee colonies, disease-free and queen-right, bred by [redacted]
Collection	With glass tubes from the flight board without the use of smoke and without anaesthetics, collected on the morning of use
Environmental conditions	Incubators
Temperature	24 - 25°C
Relative humidity	30 - 75%
Photoperiod	24 h darkness (except during observation)
Ventilation	Ventilation to avoid possible accumulation of pesticide vapour

B Study design and methods

1. In life dates	May 04 (contact test) & May 12, 2011 (2nd oral test)
2. Experimental treatments	Test units were stainless steel cages of 10 cm x 8.5 cm x 5.5 cm (length x width x height), the front side was a removable glass sheet, the bottom was perforated with 98 ventilation holes (Ø 1 mm), the inner walls were lined with filter paper. 10 bees were used per test unit, 5 replicates per test item dose level, controls and toxic standard dosages (i.e. 50 individuals per treatment group). Food was commercial ready-to-use syrup (Apiinvert; 30% Sucrose, 31% Glucose, 39% Fructose). Nominal dose level of the test item in the contact test was 100 µg a.s./bee. Nominal dose levels of the test item in the oral test were 100.0, 50.0, 25.0, 12.5 and 6.3 µg a.s./bee (dose response test).

[4] Adhäsit improves spreading of the test droplet on the water-repellent hairs on the thorax of bees

Measured dose levels of the test item in the 1st oral test were 71.5, 42.4, 28.1, 13.9 and 7.0 µg a.s./bee (dose response test).
Measured dose levels of the test item in the 2nd oral test were 101.6, 55.3, 26.3, 13.6 and 6.7 µg/L (dose response test).
Nominal dose levels of the toxic reference were 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee (contact test) and 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee (oral test). Measured dose levels of the toxic reference in the 1st oral test were 0.32, 0.16, 0.08 and 0.06 µg dimethoate/bee.
Measured dose levels of the toxic reference in the 2nd oral test were 0.27, 0.16, 0.08 and 0.05 µg dimethoate/bee.
In the contact test a CO_2/tap water + Adhaesit and a CO_2/acetone treated control were run in parallel; in the oral test a tap water/sugar and an acetone/sugar control.

Treatment groups:
Contact test: 5 replicates per treatment group (limit test)

Oral test: 1st definitive oral test: 3 replicates per treatment group (dose response test)
2nd definitive oral test: 6 replicates per treatment group (dose response test)

Application of the test item in the contact test:

Bees were anaesthetized with CO_2 in the contact test. A single 5 µL droplet of BYI 08330-mono-hydroxy (100 µg a.s./bee) in an appropriate carrier (acetone) was placed on the dorsal bee thorax using a Burkard - Applicator. For the controls, one 5 µL droplet of a) tap water containing 0.5% Adhäsit* and b) pure acetone was used. The reference item was also applied in a 5 µL droplet (dimethoate made up in acetone).

A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item; IBACON experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected; [presented as a poster on the ICPBR Bee Protection Group meeting in Bologna, 2002].

Application of the test item in the oral test:

Appropriate amounts of BYI 08330-mono-hydroxy or reference item dilutions in acetone were mixed with syrup (ready-to-use syrup: Apiinvert) in order to achieve the required test concentrations in a final dilution of 50% syrup solution (45% water, 50% syrup and 5% acetone (w/w)). For the controls, the same proportion of syrup, water and acetone was used (solvent control) and similarly, 50% aqueous syrup solution was used for the negative control.

The treated food was offered to the bees after 20 minutes starvation time in syringes which were weighed before and after introduction into the cages (duration of uptake was between 1 hour 55 minutes and 6 hours for the test item treatments). After a maximum of 6 hours the uptake was complete and syringes were removed, weighed and replaced by ones containing fresh, untreated food. The mean target dose levels (*e.g.* 100 µg a.s./bee nominal) would have been obtained if exactly 20 mg/bee of the treated food were ingested. In practice, uptake of the treated sugar solutions differed slightly from the nominal 20 mg/bee and results are given based on the measured consumption.

Test duration: 48 hours
(except 1st definitive oral test, which was prolonged to 72 hours due to increasing mortality between 24 and 48 hours)

3. Observations

The number of dead bees was assessed after 4 hours (first day); 24 and 48 hours (all tests), 72 hours (1st definitive oral test).
Behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4 hours (first day); 24 and 48 hours (all tests), 72 hours (1st definitive oral test).
Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the control in both the contact and oral tests.
The contact and oral LD50 values of the reference item were estimated using the binomial distribution (contact test) (according to [redacted], 1977) or according to moving average computations (oral test) ([redacted] and [redacted], 1952).
The LD50 calculation was carried out taking into account the mortality data corrected by control mortality using Abbott's formula (1925).
The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

RESULTS AND DISCUSSION

A. Findings

Validity of the test

The contact and oral test are considered valid as the control mortality in each case was < 10% and the LD_{50} values obtained with the reference item (dimethoate), were within the required ranges (see also tables below).

Contact Toxicity Test:

Mortality

At the end of the contact toxicity test (48 hours after application), 2% mortality occurred at 100.0 µg a.s./bee. There was also 2 % mortality in the water control group (water + 0.5 % Adhäsit) and in the solvent control group (acetone), respectively.

Behavioural abnormalities

Over the course of the contact toxicity test 3 out of the 50 bees in the test item treatment showed uncoordinated movements.
Since 2% occurred in the 100.0 µg a.s./bee group, the contact LD_{50} can be considered as > 100.0 µg a.s./bee.

Mortality and behavioural abnormalities of the bees in the contact toxicity test

	after 4 hours		after 24 hours		after 48 hours	
	mortality	behav. abnorm.	mortality	behav. abnorm.	mortality	behav. abnorm.
dosage	mean	mean	mean	mean	mean	mean
µg a.i./bee	%	%	%	%	%	%
test item						
100.0	0.0	4.0	0.0	0.0	2.0	2.0
water	2.0	0.0	2.0	0.0	2.0	0.0
solvent	0.0	0.0	2.0	0.0	2.0	0.0
reference item						
0.30	0.0	34.0	98.0	2.0	100.0	0.0
0.20	0.0	12.0	78.0	8.0	90.0	2.0
0.15	0.0	0.0	46.0	0.0	64.0	0.0
0.10	0.0	0.0	0.0	0.0	0.0	0.0

results are averages from five replicates (ten bees each) per dosage/control
see Appendix 1, Table 5 for details;
behav. abnorm. = behavioural abnormalities; water = CO_2/water-treated control; solvent = acetc

Oral Toxicity Test:

A preliminary oral limit test (GLP) with 100.0 µg a.s./bee led to 62% mortality after 48 hours. Further range finding tests (non-GLP) with 10.0 and 1.0 µg a.s./bee led to no mortality. Therefore, an oral dose response test with a maximum of 100.0 µg a.s./bee was conducted.

1st Definitive Oral Test:

The maximum nominal dose levels of spirotetramat-mono-hydroxy (100 and 50 µg a.s./bee) were not achieved, because the bees did not ingest the full volume of treated sugar solution, even when offered over a period of 6 hours. Actual oral doses of 71.5, 42.4, 28.1 and 13.9 µg a.s./bee resulted in mortality ranging from 20.0 % to 3.3% (72 hours after application). No mortality occurred in the 7.0 µg a.s./bee dose level. 3.3% mortality occurred in the solvent control group and in the water control group (50% sugar solution), respectively.

2nd Definitive Oral Test:

The bees ingested the full volume of treated sugar solution which resulted in actual doses of 101.6, 55.3, 26.3, 13.6 and 6.7 µg a.s./bee. Two out of the in total 300 test item treated bees (60 bees per treatment level, 5 treatment levels) were found dead at the end of the test. The two bees (3.3%) died in the highest dose level of 101.6 µg a.s./bee. No mortality occurred in the other dose level over the entire experimental time of 48 hours. No mortality occurred in the solvent control group and water control group (50% sugar solution), respectively

Behavioural abnormalities (1st and 2nd Definitive Oral Test)
No behavioural abnormalities attributed to exposure of the test item to the bees occurred during the entire experimental time.

Mortality (LD_{50})

1st Definitive Oral Test:
Since mortality in all of the test item dose levels was < 50%, the oral LD_{50} can be considered to be higher as the highest tested dose level (> 71.5 µg a.s./bee).

2nd Definitive Oral Test (increased number of replicates):
Since mortality in all of the test item dose levels was < 50%, the oral LD_{50} can be considered as higher as the highest tested dose level (> 101.6 µg a.s./bee).

Mortality and behavioural abnormalities of the bees in the 1st Definitive Oral toxicity test

	after 24 hours		after 48 hours		after 72 hours	
consumed dose µg a.i./bee	mortality mean %	behav. abnorm. mean %	mortality mean %	behav. abnorm. mean %	mortality mean %	behav. abnorm. mean %
test item						
71.5	10.0	0.0	20.0	0.0	20.0	0.0
42.4	3.3	0.0	3.3	0.0	3.3	0.0
28.1	6.7	0.0	6.7	0.0	6.7	0.0
13.9	3.3	0.0	3.3	0.0	3.3	0.0
7.0	0.0	0.0	0.0	0.0	0.0	0.0
water	0.0	0.0	0.0	0.0	3.3	0.0
solvent	0.0	0.0	3.3	0.0	3.3	0.0
reference item						
0.32	100.0	0.0	100.0	0.0	100.0	0.0
0.16	80.0	0.0	90.0	0.0	90.0	0.0
0.08	3.3	0.0	3.3	0.0	6.7	0.0
0.06	0.0	0.0	0.0	0.0	3.3	0.0

results are averages from three replicates (ten bees each) per dosage/control
see Appendix 1, Table 7a for details
behav. abnorm. = behavioural abnormalities

Mortality and behavioural abnormalities of the bees in the 2nd Definitive Oral toxicity test

	after 4 hours		after 24 hours		after 48 hours	
	mortality	behav. abnorm.	mortality	behav. abnorm.	mortality	behav. abnorm.
consumed dose µg a.i./bee	mean %	mean %	mean %	mean %	mean %	mean %
test item						
101.6	3.3	0.0	3.3	0.0	3.3	0.0
55.3	0.0	0.0	0.0	0.0	0.0	0.0
26.3	0.0	0.0	0.0	0.0	0.0	0.0
13.6	0.0	0.0	0.0	0.0	0.0	0.0
6.7	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
solvent	0.0	0.0	0.0	0.0	0.0	0.0
reference item						
0.27	35.0	60.0	100.0	0.0	100.0	0.0
0.16	6.7	8.3	91.7	0.0	98.3	0.0
0.08	0.0	0.0	0.0	0.0	0.0	0.0
0.05	0.0	0.0	0.0	0.0	0.0	0.0

results are averages from six replicates (ten bees each) per dosage/control
see Appendix 1, Table 7b.c for details
behav. abnorm. = behavioural abnormalities

Contact and oral (1st and 2nd Definitive Oral Test) toxicity LD_{50} values of bees treated with spirotetramat-mono-hydroxy:

	LD_{50} [µg a.s./bee]		
	Contact test	**1st Definitive Oral test**	**2nd Definitive Oral test**
Test item (48 h)	>100.0	> 71.5	> 101.6
Toxic standard (24 h) (95% Confidence limit)	0.16 (0.10 - 0.20)	0.12 (0.08 - 0.16)	0.12 (0.11 - 0.13)

B. Observations

Over the course of the contact toxicity test 3 out of the 50 bees in the test item treatment showed uncoordinated movements.

No behavioural abnormalities attributed to exposure of the test item to the bees occurred during the experimental time of 48 (72) hours for the oral toxicity test (both oral tests).

CONCLUSION

The toxicity of spirotetramat-mono-hydroxy was tested in both, an acute contact limit test and in two acute oral toxicity dose response tests on honey bees. The LD_{50} (24 + 48 h) of spirotetramat-mono-hydroxy was determined to be > 100.0 µg a.s./bee in the contact toxicity test.

The data obtained from the two independent definitive oral toxicity dose-response tests are in line, and based on the results of both dose-response test runs, the oral LD_{50} (24 + 48 h) can be concluded to be > 101.6 µg a.s./bee.

IIA 8.7.2 Acute contact toxicity

A study with spirotetramat on acute contact toxicity is summarised in IIA 8.7.1/01.

IIA 8.7.3 Toxicity of residues on foliage to honey bees*

The acute toxicity to bees is not sufficient to require this study in the U.S. Furthermore, higher tier studies (semifield/field studies) are available in which effects of aged foliar residues to bees were observed over several weeks and are presented in respective Annex III dossiers.

IIA 8.7.4 Bee brood feeding test

Report:	**KIIA 8.7.4/01, [redacted]; 2004**
Title:	Assessment of the Side-Effects of BYI 08330 OD 100 on the Honey Bee (*Apis mellifera* L.) in a Feeding Test. Date: 2004-02-23
Testing facility/Data Owner:	GAB Biotechnologie & IFU Umweltanalytik GmbH, Germany [redacted], Germany
Report No.:	20031168/01-BFEU; M-000345-01-2
Publication:	Unpublished
Dates of experimental work:	May 23, 2003-June 13, 2003
Guidelines:	OEPP/EPPO Bulletin No. 22 (Oomen et al, 1992)
Deviations:	no major deviations
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the toxicity of Spirotetramat (BYI 08330) OD 100 to Honey bees (*Apis mellifera* L.) in a feeding study under field conditions. Colonies of honey bees were fed with the test substance (dissolved in water and mixed in pure liquid sucrose solution at a concentration of 0.0144% a.s. of BYI 08330 OD 100). The control group was fed with pure sugar solution and the toxic standard treatment with 300 mg Insegar 25 WG per colony (dissolved in water and mixed in pure liquid sucrose solution). Each treatment contained 3 replicates (bee colonies). Mortality, flight intensity, condition of the colonies and the development of the bee brood was assessed before and after the feeding of the test substance. Particular attention was directed to the brood development of the colonies. It was concluded that the feeding of Spirotetramat OD 100 had no effect on the mortality of the bees and did not result in an adverse effect on the flight activity and the behaviour of the bees in front of the

* No EC data requirement (the OECD point concerned is not covered by or part of an EC point according to Council Directive 91/414/EEC. Hence, data/documents do not need to be submitted.

hives. Regarding the brood development a high termination rate was noticed 4 days after start of feeding in two of the three test substance colonies. In these colonies the further brood development was massively disturbed at the following assessments and they were not able to compensate the effect on the brood development caused by the test substance Spirotetramat OD 100 until the end of the observation period. No effect was detected by the regular brood assessments.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat OD 100 (a.s. BYI 08330)**
Description	**liquid, brownish suspension**
Lot/batch No.	**Batch no.: 08030/0038(0035)**
	Tox no.: 06293-00
Content a.s.	**101.54 g/L**
Stability of test compound	**Test item is considered sufficiently stable in spray solution (at least 1 hour)**
2. Vehicle and/or positive control	**Water/sucrose solution** **300 mg Insegar 25 WG per colony (active substance: fenoxycarb, 25%)**
3. Test animals	
Species	**Honey bee (*Apis mellifera* L.), healthy queen-right bee colonies with 10 combs (one brood body). Appr: 12000 bees per colony**

B Study design and methods

1. In life dates **May 23, 2003 – June 13, 2003**

2. Experimental treatments

The feeding test was located in Southern Germany near [redacted] (location: [redacted]). All colonies of the study were placed at one location. Bees from the test colonies were free-flying, with access to natural nectar sources. No main sources of nectar were found in the near surroundings of the test colonies. The health of the colonies (e.g. *Varroa*) was checked before the initiation of the test. All colonies were in good condition at start of the test. Colonies of honey bees were fed with the test substance BYI 08330 OD 100 (dissolved in water and mixed in pure liquid sucrose solution at a concentration of 0.0144% a.s. of BYI 08330 OD 100 shortly before feeding). The control group was fed with pure sugar solution and the toxic standard treatment with 300 mg Insegar 25 WG per colony (dissolved in water and mixed in pure liquid sucrose solution). Each treatment contained 3 replicates (bee colonies). A quantity of 1 kg sugar solution was offered per colony.

3. Observations

Mortality, flight intensity, condition of the colonies and the development of the bee brood was assessed before and after the feeding of the test substance. Particular

attention was directed to the brood development of the colonies. Wooden bee traps (Münster design) with gauze on bottom and on 100% of the top were attached to the entrance of the colonies in order to register those dead bees which were carried out of the hives. The mortality of adult honey bees and bee brood in the bee trap was assessed separately for each colony. Evaluation of mortality took place over three days before start of feeding (once a day at the same time in the morning), at the day of start of feeding (twice before start of feeding) and for 21 days after start of feeding (once a day at the same time in the morning). The influence of the test substance BYI 08330 OD 100 was evaluated by comparing the results in the test substance treatment to the control data and those from the toxic standard treatment and furthermore by comparing the pre- and post-feeding results in view of the following observations:

- **Adult honey bee, pupae and larvae mortality in the bee traps**
- **Condition of the colonies**
- **Development of the bee brood**
- **Behaviour of the bees around the hive**

RESULTS AND DISCUSSION

A. Findings

The test was considered to be valid because the following parameters were met.

A detectable effect of the toxic standard was found (brood termination before a successful hatch, mortality of pupae)

A clear difference was found in the brood termination rate in cells between the colonies in the control and the colonies in the toxic standard treatment.

Mortality and flight activity of honey bees in the feeding study

Test substance		Spirotetramat OD 100		
Test object		*Apis mellifera*		
Exposure		Feeding of the colonies with the test substance diluted in pure sugar solution		
Treatment group		Test substance (BYI 08330 OD 100)	Control (pure sugar solution)	Toxic standard (Insegar 25 WG)
Feeding rate per colony (in pure sugar solution)		0.0144% a.s.	-	300 mg product
Average Mortality [dead bees/ colony/day]	pre (DAF –3 to 0bf):	6.8	7.1	7.2
	post ± SD (DAF 1 to 21)	10.4±11.6	4.9±4.0	91.2±106.5
	$QM_{(average)}$:	1.5	0.7	12.7
Σ dead adult bees and dead pupae (post-feeding, DAF 1 to 21)		597 + 55P	282 + 29P	321 + 5425P
Average Flight activity*	0bf	46.67	28.67	38.00
	DAF 1	57.00	35.00	57.00
	DAF 3	17.67	23.33	28.00

DAF		days after start of feeding
0bf		mortality on the day of start of feeding before feeding
QM (average)		∅ post-feeding mortality ÷ ∅ pre-feeding mortality
Pre		average value for the pre-feeding period DAF –3 to 0bf
post (DAF 1 to 21)		average value for the post-feeding period DAF1 to 2
P	=	dead pupae
SD	=	Standard deviation

* mean number of bees leaving/entering the hive per minute

Regarding the total daily mortality in the test substance treatment a slightly increased mean mortality was noticed in the test substance group on DAF 6, 13 and 15 with 24.7 to 32.0 dead bees/colony. Throughout the entire post-feeding period a sum of 55 dead pupae were counted in the test substance treatment compared to 29 dead pupae in the control group. A sum of 5425 dead pupae were counted during the entire post-feeding period in the toxic standard which is evidence that Insegar 25 WG massively disturbed the brood development of these test colonies. The total number of dead adult bees during the post-feeding period was 597 dead bees in the test substance treatment, 282 dead adult bees in the control and 321 dead adult bees in the toxic standard treatment.
The mean post-feeding mortality (adult bees and pupae) was determined as 4.9 dead bees/day/colony in the control treatment, 10.4 dead bees/day/colony in the test substance treatment and 91.2 dead bees/day/colony in the toxic standard treatment.

B. Observations

Honey bee flight intensity:

Regarding the flight intensity observed in front of the hives no remarkable differences were observed at both post-feeding assessment dates DAF 1 and 3 between the control, the test substance and the toxic standard treatment.

Condition of the colonies:

During the assessments of the condition of the colonies throughout the study no remarkable observations were made regarding the strength of the colonies and the brood nest size in the test substance treatment compared to the control treatment. The colonies of the test substance treatment, control and toxic standard treatment showed all brood stages at the assessment dates during the experimental phase of the study.

Honey bee brood development:

By comparing the individual brood assessments of single cells, the indices (the values of the different brood stages of all cells in each treatment group, assessed at the same date, summed up and divided by the number of observed cells) of the control group showed the course to be expected in a natural bee development cycle. In hive 2 and 3 of the test substance treatment a termination of the bee brood in more than three quarters of the cells observed was noticed from BFD (brood fixing day) to BFD 5. The cells which showed a terminated brood development were empty at the first assessment after start of feeding of BYI 08330 OD 100. Even at the following assessments a massively disturbed brood development was observed with empty cells, cells refilled or refilled again with new brood. Hive 2 and 3 of the test substance treatment were not able to compensate the effect on the brood development caused

by the test substance BYI 08330 OD 100 until the end of the observation period. No clear effect which could be attributed to the test substance was observed at hive 1.

Honey bee behaviour in front of the colonies:

No differences regarding the behaviour of the bees were noticed between the test substance treatment, the toxic standard treatment and the control.

CONCLUSION

It was concluded that the feeding of BYI 08330 OD 100 (dissolved in water and mixed in pure liquid sucrose solution at a concentration of 0.0144% a.s. of BYI 08330) had no effect on the mortality of the bees and did not result in an adverse effect on the flight activity and the behaviour of the bees in front of the hives. Regarding the brood development a high termination rate was noticed 4 days after start of feeding in two of the three test substance colonies. In these colonies the further brood development was massively disturbed at the following assessments and they were not able to compensate the effect on the brood development caused by the test substance BYI 08330 OD 100 until the end of the observation period. No effect was detected by the regular brood assessments.

Report:	**KIIA 8.7.4/02 [redacted]; 2004**
Title:	Assessment of the Side-Effects of BYI 08330 SC 240 on the Honey Bee (*Apis mellifera* L.) in a Feeding Test. Date: 2004-02-05
Testing facility/Data Owner:	GAB Biotechnologie & IFU Umweltanalytik GmbH, Germany [redacted], Germany
Report No.:	20031166/01-BFEU; M-121877-01-2
Publication:	unpublished
Dates of experimental work:	May 23, 2003 -June 13, 2003
Guidelines:	OEPP/EPPO Bulletin No. 22 (Oomen et al, 1992)
Deviations:	no major deviations
GLP:	yes (certified laboratory)

Executive summary

The objective of the study was to determine the effects of BYI 08330 SC 240 on the honey bee, *Apis mellifera* L. in a feeding study under field conditions. Colonies of honey bees were fed with the test substance BYI 08330 SC 240 (dissolved together with the adjuvant in water and mixed in pure liquid sucrose solution), pure sugar solution for the untreated control treatment and Insegar 25 WG (a.s.: fenoxycarb, 25%) in the toxic standard treatment (dissolved in water and mixed in pure liquid sucrose solution). Each treatment contained 3 replicates (bee colonies). Mortality, flight intensity, condition of the colonies and the development of the bee brood was assessed before and after the feeding of the test substance. Particular attention was directed to the brood development of the colonies. It was concluded that the feeding of BYI 08330 SC 240 (dissolved in water and mixed in pure liquid sucrose solution at a concentration of 0.0144% a.s. of BYI 08330 SC 240 plus 0.200% a.s. of the adjuvant RME EW 500) caused an increase of adult honey bee mortality 6 and 15 to 17 days after start of feeding but did not result in an adverse effect on the flight activity and the behaviour of the bees in front of the hives. An

almost total termination of the brood development was noticed 4 days after start of feeding in all colonies but the colonies showed the tendency to recover until the end of the observation period since most of the cells observed contained developing brood at the final brood assessment and no effect was detected by the regular brood assessments.

MATERIAL AND METHODS

A Materials

1. Test material	**BYI 08330 SC 240**
Description	**Liquid, white**
Lot/batch No.	**Batch no.: 07218/0013**
	Tox no.: 06294-00
Content a.s.	**243.85 g/L (analysed)**
Stability of test compound	**sufficiently stable in spray solution (at least 1 hour)**
2. Adjuvant	**RME EW 500 (rape methyl ester)**
Description	**Liquid/white**
Lot/batch No.	**Batch no.: 05778/0209**
	Tox no.: 06355-00
Content a.s.	**500 g/L**
Stability of test compound	**sufficiently stable in spray solution (at least 1 hour)**
3. Vehicle and/or positive control	**Water/sucrose solution**
	300 mg Insegar 25 WG per colony (active substance: fenoxycarb, 25%)
4. Test animals	
Species	**Honey bee (*Apis mellifera* L.), healthy queen-right bee colonies with 10 combs (one brood body). Approx. 12 000 bees per colony**

B Study design and methods

1. In life dates **May 23, 2003 – June 13, 2003**

2. Experimental treatments

The feeding test was located in Southern Germany near [redacted] (location: [redacted]). All colonies of the study were placed at one location. Bees from the test colonies were free-flying, with access to natural nectar sources. No main sources of nectar were found in the near surroundings of the test colonies. The health of the colonies (e.g. *Varroa*) was checked before the initiation of the test. All colonies were in good condition at start of the test. The test substance solution containing BYI 08330 SC 240 was prepared shortly before feeding at the test concentration of 0.0144% a.s. and with 0.200% a.s. of the adjuvant RME EW 500 in 1 L sucrose solution per colony.

The test substance BYI 08330 SC 240 and the adjuvant RME EW 500 were first dissolved in water. Afterwards the water-test substance solution was mixed with a definite amount of a pure liquid sucrose solution (Api-Invert) in order to get the feeding solution. The colonies of the untreated control treatment were fed with pure sugar solution and in the toxic standard treatment with sucrose solution containing Insegar 25 WG (Insegar 25 WG dissolved in water and mixed with pure liquid sucrose solution).

A quantity of 1 kg of the test solution was offered by placing a feeding container with the test solution into each colony. The feeding was started simultaneously for all colonies and lasted until the solution was consumed.

3. Observations

Mortality, flight intensity, condition of the colonies and the development of the bee brood was assessed before and after the feeding of the test substance. Particular attention was directed to the brood development of the colonies. Wooden bee traps (Münster design) with gauze on bottom and on 100% of the top were attached to the entrance of the colonies in order to register those dead bees which were carried out of the hives. The mortality of adult honey bees and bee brood in the bee trap was assessed separately for each colony. Evaluation of mortality took place over three days before start of feeding (once a day at the same time in the morning), at the day of start of feeding (twice before start of feeding) and for 21 days after start of feeding (once a day at the same time in the morning). The influence of the test substance BYI 08330 SC 240 was evaluated by comparing the results in the test substance treatment to the control data and those from the toxic standard treatment and furthermore by comparing the pre- and post-feeding results in view of the following observations:

- **Adult honey bee, pupae and larvae mortality in the bee traps**
- **Condition of the colonies**
- **Development of the bee brood**
- **Behaviour of the bees around the hive**

RESULTS AND DISCUSSION

A. Findings

The test was considered to be valid because the following parameters were met:
A detectable effect of the toxic standard was found (brood termination before a successful hatch, mortality of pupae)
A clear difference was found in the brood termination rate in cells between the colonies in the control and the colonies in the toxic standard treatment.

Mortality and flight activity of honey bees in the feeding study

Test substance		BYI 08330 SC 240		
Test object		*Apis mellifera*		
Exposure		Feeding of the colonies with the test substance diluted in pure sugar solution		
Treatment group		Test substance + Adjuvant (BYI 08330 SC 240 + RME EW 500)	Control (pure sugar solution)	Toxic standard (Insegar 25 WG)
Feeding rate per colony (in pure sugar solution)		0.0144% a.s. + 0.200% a.s.	-	300 mg product per colony
Average Mortality	pre (DAF –3 to 0bf):	6.9	7.1	7.2

[dead bees/ colony/day]	post ± SD (DAF 1 to 21)	15.8±24.1	4.9±4.0	91.2±106.5
	$Q_{M(average)}$:	2.3	0.7	12.7
∑ dead adult bees and dead pupae (post-feeding, DAF 1 to 21)		988 + 10P	282 + 29P	321 + 5425P
Average Flight activity*	0bf	36.33	28.67	38.00
	DAF 1	45.00	35.00	57.00
	DAF 3	19.00	23.33	28.00

DAF days after start of feeding
0bf mortality on the day of start of feeding before feeding
Q_M (average) ∅ post-feeding mortality ÷ ∅ pre-feeding mortality
Pre average value for the pre-feeding period DAF –3 to 0bf
post (DAF 1 to 21) average value for the post-feeding period DAF 1 to 21
P = dead pupae
SD = Standard deviation
* mean number of bees leaving/entering the hive per minute

Regarding the total daily mortality in the test substance treatment an increased daily mean mortality was noticed on DAF 6 as well as on DAF 15 to 17 with 23.3 to 71.0 dead bees/colony. Only 10 dead pupae were counted in the test substance treatment throughout the entire post-feeding period compared to 29 dead pupae in the control group. A sum of 5425 dead pupae were counted during the entire post-feeding period in the toxic standard which is evidence that Insegar 25WG massively disturbed the brood development of these test colonies. The total number of dead adult bees during the post-feeding period was 988 dead bees in the test substance treatment, 282 dead adult bees in the control and 321 dead adult bees in the toxic standard treatment.
The mean post-feeding mortality (adult bees and pupae) was determined as 4.9 dead bees/day/colony in the control treatment, 15.8 dead bees/day/colony in the test substance treatment and 91.2 dead bees/day/colony in the toxic standard treatment.

B. Observations

Honey bee flight intensity:
Regarding the flight intensity observed in front of the hives no remarkable differences were observed at both post-feeding assessment dates DAF 1 and 3 between the control, the test substance and the toxic standard treatment.

Condition of the colonies:
During the assessments of the condition of the colonies throughout the study no remarkable observations were made regarding the strength of the colonies and the brood nest size in the test substance treatment compared to the control treatment. The colonies of the test substance treatment, control and toxic standard treatment showed all brood stages at the assessment dates during the experimental phase of the study.

Honey bee brood development:
By comparing the individual brood assessments of single cells, the indices (the values of the different brood stages of all cells in each treatment group, assessed at the same date, summed

up and divided by the number of observed cells) of the control group showed the course to be expected in a natural bee development cycle. In all colonies of the test substance treatment a termination of the brood in almost all cells was noticed from BFD (Brood area fixing day) to BFD 5. Hive 1 and 2 were able to compensate this lack of brood and the main part of the empty cells was filled again with eggs and larvae at the following assessment on BFD 11. In hive 3 of the BYI 08330 SC 240 treatment the situation of the marked brood area was similar on BFD 11 compared to the previous assessment because the cells remained empty until this assessment day and even at BFD 15 the brood index was still below the level of 1 (egg stage). These observations indicate an effect of BYI 08330 SC 240 of to the brood development in the first 5 to 15 days after start of feeding. Nevertheless it should be considered that the brood indices continued to rise from BFD 11 to 22 in all test substance colonies and that at the final brood assessment the big part of the cells contained brood.

Honey bee behaviour in front of the colonies:

No differences regarding the behaviour of the bees were noticed between the test substance treatment, the toxic standard treatment and the control. Honey bee flight intensity:

Regarding the flight intensity observed in front of the hives no remarkable differences were observed at both post-feeding assessment dates DAF 1 and 3 between the control, the test substance and the toxic standard treatment.

CONCLUSION

It was concluded that the feeding of BYI 08330 SC 240 (dissolved in water and mixed in pure liquid sucrose solution at a concentration of 0.0144% a.s. of BYI 08330 SC 240 plus 0.200% a.s. of the adjuvant RME EW 500) caused an increase of adult honey bee mortality 6 and 15 to 17 days after start of feeding but did not result in an adverse effect on the flight activity and the behaviour of the bees in front of the hives. An almost total termination of the brood development was noticed 4 days after start of feeding in all colonies but the colonies showed the tendency to recover until the end of the observation period since most of the cells observed contained developing brood at the final brood assessment and no effect was detected by the regular brood assessments.

IIA 8.8 Effects on non-target terrestrial arthropods

IIA 8.8.1 Effects on non-target terrestrial arthropods, artificial substrates

IIA 8.8.1.1 Parasitoid

Report:	**KIIA 8.8.1.1/01, [redacted]; 2005**
Title:	Toxicity to the parasitoid wasp *Aphidius rhopalosiphi* ([redacted]) (Hymenoptera: Braconidae) in the laboratory BYI 08330150 OD
	Date: 2005-07-15
Organisation:	Bayer CropScience GmbH, Frankfurt, Germany

Report No.:	CW05/023; M-25442-01-1
Publication:	Unpublished
Dates of experimental work:	April 18, 2005 – May 04, 2005
Guidelines:	IOBC (Mead-Briggs et al. 2000)
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the toxicity of freshly dried residues applied onto glass plates to the parasitoid wasp *Aphidius rhopalosiphi*. Spirotetramat OD 150 was applied at rates of 2; 9; 24; 70 and 200 g a.s./ha, and the effects were compared to a toxic reference (a.s.: dimethoate) applied at 0.1 g a.s./ha and a water treated control. Mortality of 60 adults was assessed 24 and 48 hours after exposure.

In the highest tested dose rate of 200 g a.s./ha 85.5% of corr. mortality was observed. At the lower rates of 70 and 24 g a.s./ha 14.5% and 0% corr. mortality were detected and -7.3 and -3.6% in the 9 and 2 g a.s./ha rate. The LR_{50} (median lethal rate) was determined to be 114.7 g a.s./ha.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) 150 OD**
Description	**Not specified**
Lot/batch No.	**Batch no.: 08030-0189(0152)** **Tox no.: TOX07034-00**
Analytical content	**148.89 g a.s./L**
Stability of test compound	**Stability of the test item is guaranteed in the certificate of analysis, 2003-03-10**
2. Vehicle and/or positive control	**Deionised water** **Dimethoate: 392.1 g/L = 36.82% w/w**
3. Test animals	
Species	**Parasitoid wasps *Aphidius rhopalosiphi***
Age	**< 48 h**
Source	**[redacted], Germany**
Acclimation period	**Until the start of the study, the mummies were stored at the temperature given by the breeder. 2 days prior to the start of the study, all hatched animals were removed from the tubes to ensure that the age of the test animals was below 48 h.**
Environmental conditions	
Temperature	**19 - 22°C**
Photoperiod	**16:8 hours ,** **570 - 1001 Lux (measured once)**

B Study design and methods

1. In life dates **April 18, 2005 – May 04, 2005**

2. Experimental treatments

15 *Aphidius rhopalosiphi* per replicate were exposed to glass plates treated with 2; 9; 24; 70 and 200 g a.s./ha (4 replicates per concentration) for 2 days. The effects were compared to a toxic reference (a.s.: dimethoate) applied at 0.1 g a.s./ha and a water treated control.

The test units consisted of two treated glass plates (100 x 100 x 3 mm) and an untreated acryl frame (inner size 92 x 92 x 14 mm) with 3 ventilation holes on each side. These holes were covered with gauze (mesh size 80 µm) which was jammed in the orifices by pieces of Teflon tubing (8 mm outer diameter). The test animals were introduced from prepared test tubes by slightly tapping and/or darkening them.

If all wasps were in the test unit, the glass tube was removed and the orifice closed with a feeding stopper. During the exposure phase of 2 days the test animals had access to the sugar solution.

3. Observations

At the end of the exposure period the condition of the test animals was recorded:

- 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)

and the mortality was assessed.

The corrected mortality was obtained by comparing the values observed in the treated samples with those in the control samples, according to the formula of ABBOTT's (1925). The computer program SAS was used to perform the statistical analyses.

RESULTS AND DISCUSSION

A. Findings

After 48 hours of the test 8.3% of the wasps were found dead in the control group. In the reference item group, 100% of the wasps were dead after 24 hours of exposure. Thus, the validity criteria of the study were met and the results can be considered as valid.

Test item	Spirotetramat OD 150		
Test object	*Aphidius rhopalosiphi*		
	Mortality [%] – 48 hours after treatment		
Treatment	Uncorr.	Abbott	P-Value(*)
Control (deionised water)	8.3	-	
2 g a.s./ha	5.0	-3.6	n.sign.
9 g a.s./ha	1.7	-7.3	0.620 n.sign.
24 g a.s./ha	8.3	0	n.sign.
70 g a.s./ha	21.7	14.5	0.28 n.sign.
200 g a.s./ha	86.7	85.5	0.0001 sign.
Reference item 0.1 g a.s./ha	100	100	
LR_{50} (95% CI)	114.676 g a.s./ha (86.182 – 145.165)		

* Fisher's Exact test, two-sided, p-values are adjusted according to Bonferroni-Holm
sign. = significant; n.sign. = not significant

B. Observations

In this laboratory test the effects of BYI 08330 150 OD residues on the survival of *Aphidius rhopalosiphi* were determined at rates of 2; 9; 24; 70 and 200 g a.s./ha, applied to glass plates. In the highest dose rate of 200 g a.s./ha 85.5% of corr. mortality was observed. At the lower rates of 70 and 24 g a.s./ha 14.5% and 0% corr. mortality were detected and -7.3 and -3.6% in the 9 and 2 g a.s./ha rate.

CONCLUSION

The LR_{50} was calculated to be 114.7 g a.s./ha.

IIA 8.8.1.2 Predatory mites

Report:	**KIIA 8.8.1.2/01, [redacted]; 2005**
Title:	Toxicity to the predatory mite *Typhlodromus pyri* SCHEUTEN (Acari, Phytoseiidae) in the laboratory BYI 08330 150 OD Date: 2005-07-12
Organisation:	Bayer CropScience GmbH, Frankfurt, Germany
Report No.:	CW05/024; M-254269-01-1
Publication:	Unpublished
Dates of experimental work:	May 03, 2005 – May 10, 2005
Guidelines:	IOBC(Blümel et al. 2000)
Deviations:	very short decline in rel. humidity
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the toxicity of freshly dried residues applied onto glass cover slides to the predatory mite *Typhlodromus pyri*. The test item was applied at rates of 0.19; 0.43; 0.97; 2.21 and 5.00 g a.s./ha and the effects were compared to a toxic reference (a.s.: dimethoate) applied at 4 g a.s./ha, and a water treated control. Mortality of 100 protonymphs was assessed 1, 3 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. In the three highest dose rates of 0.97; 2.21 and 5.00 g a.s./ha BYI 08330 150 OD there was 100% corr. mortality. At the lower rates of 0.43 and 0.19 g a.s./ha of the test item 71.1 and 10.3% corr. mortality were found. The LR_{50} was calculated to be 0.333 g a.s./ha.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) 150 OD**
Description	**Not specified in the report**
Lot/batch No.	**Batch no.: 08039/0189(0152)**
	Tox no.: TOX07034-00
Analytical content	**148.89 g a.s./L**
Stability of test compound	**The stability of the test item BYI 08330 150 OD and the reference item is guaranteed in the certificate of analysis.**
2. Vehicle and/or positive control	**Deionised water**
	Dimethoate: 392.1g/L = 36.82% w/w
3. Test animals	
Species	**Predatory mite *Typhlodromus pyri***
Age	**Protonymphs**
Source	**Eggs of the predatory mite *Typhlodromus pyri* were supplied by [redacted], Germany**

Acclimation period	**The test animals were kept under conditions recommended by the supplier until the start of the test.**
Environmental conditions	
Temperature	**24 - 25.5°C**
Photoperiod	**16:8 hours , 795 - 1060 lux (measured once)**

B Study design and methods

1. In life dates **May 03, 2005 – May 10, 2005**

2. Experimental treatments

One exposure unit consisted of two cover slides (glass) which were put together at the longer sides with a narrow gap between the slides. Cover slides were placed on tissue paper which again was placed on top of a wet foam rubber. To avoid the escape of the mites a barrier of sticky material was added. This test arena was transferred to a plastic tray (Bellaplast Polar Cup) filled with deionised water. The narrow gap between the two cover slides was filled with water by capillary forces to provide the mites with water. The test units were prepared one day before application to make sure that the foam rubber was saturated with water.

The test concentrations and the suspension for the reference were prepared stepwise on the day of application. Deionised water was used as diluent for both items. The items were applied to the test cover glasses using a sprayer.

In total, 3 treatments were established (control, reference item and test item with 5 rates). Each treatment was replicated 5 times with 20 mites per replicate.

After the test units were set up (day 0) the protonymphs were placed onto the glass surface. The mites were transferred with a fine brush under a stereomicroscope and immediately afterwards examined to ensure they were undamaged and in good health. Then pollen (birch – pine mixture) was supplied as food and the units were kept under test conditions.

3. Observations

On days 1, 3 and 7 the number of dead and living mites was counted. Dead mites were removed and the number of escaped mites was calculated.

On day 7 the number of females, males, eggs and juveniles was counted.

On day +7 of each test, the number of dead and escaped mites was summed up for each replicate and calculated as percentages. A mean value of the four replicates was calculated.

The corrected mortality was obtained by comparing the values observed in the treated samples with those in the control samples, according to the formula of Abbott's (1925).

The computer program SAS was used to perform the statistical analyses.

RESULTS AND DISCUSSION

A. Findings

The results can be considered as valid as the mortality/escaping rate in the control chambers up to day 7 after treatment was 3.0% and the average mortality (dead and escaped mites = Mort/Esc-value) in the reference item was > 50%.

The mean corrected mortality of the nymphs exposed to the test item and the toxic reference is given in the table below:

Test item	Spirotetramat OD 150		
Test object	*Typhlodromus pyri*		
	Mortality [%] - 7 days after treatment		
Treatment	Uncorr.	Abbott	P-Value(*)
Control (deionised water)	3.0	0	-
0.19 g a.s./ha	13.0	10.3	0.016 sign.
0.43 g a.s./ha	72.0	71.1	<.0001 sign.
0.97 g a.s./ha	100	100	<.0001 sign.
2.21 g a.s./ha	100	100	<.0001 sign.
5.00 g a.s./ha	100	100	<.0001 sign.
Reference item 4 g a.s./ha	88.0	87.6	-
LR_{50} (95% CI)	0.333 g a.s./ha (0.298 – 0.367)		

* Fisher's Exact test, two-sided, p-values are adjusted according to Bonferroni-Holm
sign. = significant

B. Observations

In this laboratory test the effects of BYI08330 150 OD residues on the survival of the predatory mite *Typhlodromus pyri* were determined at the rates of 0.19; 0.43; 0.97; 2.21 and 5.00 g a.s./ha applied to glass cover slides.
In the three highest dose rates of 0.97; 2.21 and 5.00 g a.s./ha BYI 08330 150 OD there was 100% corr. mortality. At the lower rates of 0.43 and 0.19 g a.s./ha of the test item 71.1 and 10.3% corr. mortality were found.

CONCLUSION

The LR_{50} was calculated to be 0.333g a.s./ha.

IIA 8.8.1.3 Ground dwelling predatory species

Covered by laboratory and extended laboratory studies on sensitive leaf-dwelling species: since spirotetramat will be applied to crops and not to bare ground, exposure of foliage-dwelling species can be considered to be more severe than in ground-dwelling species.

IIA 8.8.1.4 Foliage dwelling predatory species

Not required as extended laboratory studies are available for foliage dwelling predators.

IIA 8.8.2 Effects on non-target terrestrial arthropods in extended lab/semi-field test

IIA 8.8.2.1 Parasitoid

Report:	**KIIA 8.8.2.1/01, [redacted]; 2006**
Title:	Toxicity to the parasitoid wasp *Aphidius rhopalosiphi* ([redacted]) (Hymenoptera: Braconidae) using an extended laboratory test; BYI 08330 150 OD. Date: 2006-01-18
Organisation:	Bayer CropScience GmbH, Frankfurt, Germany
Report No.:	CW05/071; M-264179-01-1
Publication:	Unpublished
Dates of experimental work:	October 17, 2005 - October 31, 2005
Guidelines:	IOBC (Mead-Briggs et al. 2000); Mead-Briggs et al. 2002
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to investigate the lethal and sublethal toxicity of freshly dried residues applied onto barley plants to the parasitoid wasp *Aphidius rhopalosiphi*. The test item was applied at rates of 22, 42, 80, 151 and 288 g a.s./ha, and the effects were compared to a toxic reference (a.s.: dimethoate) applied at 2.5 g a.s./ha and a water treated control. Mortality of 30 females was assessed 2, 24 and 48 hours after exposure. Repellency of the test item was determined during the initial 2 h after the release of the females. Five separate observations were made at 30-minute intervals starting immediately after the introduction of all wasps. From the water control and the dose rates 80, 151 and 288 g a.s./ha, 15 impartially chosen females per treatment were each transferred to a cylinder containing untreated cereal plants infested with *Rhopalosiphum padi* for a period of 24 hours. The number of mummies was assessed 11 days later.

In the dose rates of 80 and 288 g a.s./ha 0% of mortality was observed. At the rates of 22 and 42 g a.s./ha 3.3% mortality were detected and 6.7% in the 151 g a.s./ha rate. There was no reduction in reproductive success relative to the control at the 288; 151 and 80 g a.s./ha rates. No dose related repellent effect of the test item was observed. The LR_{50} was estimated to be > 288 g a.s./ha..

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) 150 OD (product code: AE 1302943 00 OD15 A101)**
Description	**Not specified in the report**
Lot/batch No.	**Batch no.: 08030/0189** **Tox no.: TOX07034-00; (0152)**
Analytical content	**148.89 g a.s./L**
Stability of test compound	**Stability of the test item is guaranteed in the certificate of analysis.**

2. Vehicle and/or positive control	**Deionised water** **Dimethoate: 392.1 g/L = 36.82% w/w**
3. Test animals	
Species	**Parasitoid wasp *Aphidius rhopalosiphi***
Age	**< 48 h**
Source	***Aphidius rhopalosiphi* and *Rhopalosiphum padi* were provided by [redacted]. Cereal plants were provided by the Global Biology Herbicide group.**
Acclimation period	**Until the start of the study, the mummies were stored at the temperature given by the breeder. 2 days prior to the start of the study, all hatched animals were removed from the tubes to ensure that the age of the test animals was below 48 h. The animals were fed via feeding tubes. The feeding solution consisted of 3 parts of water + 1 part of honey.**
Environmental conditions	
Temperature	**19-23.5°C**
Photoperiod	**16:8 hours (light:dark); 638-2990 lux in the mortality phase, 688-1718 lux in the parasitation phase and 5590-19730 lux in the reproduction phase of the study.**

B Study design and methods

1. In life dates **October 17, 2005 – October 31, 2005**

2. Experimental treatments

For the mortality assessment 7 days prior to the start of the study, the barley plants were sown (10 seed grains each). The plants were trimmed to a uniform height of 10-12 cm tall prior to the test commencing. For the reproduction assessment 5 days prior to the start of the study, the oat plants required were sown (approx. 20 seed grains each). 1 day after the start of the study, the plants were infected with *Rhopalosiphum padi*. Prior to application, the plants were sprayed with a 10% fructose solution and were left to dry for at least 1 hour. After spraying the plants with fructose solution the soil surface was covered with a thin layer of quartz sand before treatment.

The suspensions for the test and reference substances were prepared on the day of application. They were applied to the test plants using a sprayer

The test units consisted of a pot with treated barley plants which were enclosed within a clear polyacrylic cylinder (195 mm high and 100 mm in diameter) with a hole (approximately 5 mm in diameter) for the introduction of the parasitoids. After introduction the hole was closed by a stopper. The top of the cylinder was closed with fine mesh gauze. The test animals were introduced from prepared test tubes by

slightly blowing them. If all wasps were in the test unit, the glass tube was removed and the orifice closed with a feeding stopper. During the exposure phase of 2 days the test animals had access to the sugar solution. In total, 6 replicates were established, each with 5 individuals of *Aphidius rhopalosiphi*.

3. Observations

To determine whether residues of the test item were repellent to the wasps, observations on the position of the individual insects were made during the initial 2 hours after their release. 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 plants), the cylinder (on the walls of the test arena) or the soil (on the sand below the plants). Wasps on the plants have not been counted directly, but the number of wasps on the cylinder and soil was subtracted from the number of introduced wasps.

At the end of the exposure period the condition of the test animals was recorded:

- 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)

and the mortality was assessed. The corrected mortality was obtained by comparing the values observed in the treated samples with those in the control samples, according to the formula of [redacted] (1947).

Subsequently 15 healthy females were transferred and kept individually in untreated acrylic cylinders with aphid-infested cereal plants. One day later the wasps were removed and the plants kept for 11 more days. The cylinders were aerated to avoid the formation of condensed water on the walls. The parasitation rate was determined by counting the number of mummies for each individual wasp. The computer program SAS was used to perform the statistical analyses.

RESULTS AND DISCUSSION

A. Findings

The results can be considered as valid, as all validity criteria of the test were met (mortality in water control 0%, corrected mortality reference item 80%, mean reproduction per female in water control 23, no more than 2 wasps producing zero reproduction in water control).

Mortality and reproduction after exposure of *Aphidius rhopalosiphi* to freshly dried residues of Spirotetramat OD 150 applied onto barley plants

	Mortality			Reproduction			Repellency	
Treatment	**Uncorr.**	**Abbott**	**Rate**	**Rel. to control [%]**	**P-value ***	**% wasps on plant**	**Rel. to control [%]**	**P-value ***
Control	**0**	**0**	**23**	**0**	**-**	**75.3**	**-**	**-**
22 g a.s./ha	**3.3**	**3.3**	**n.d.***	**n.d.***	**-**	**75.1**	**0.4**	**1 (n.s.)**
42 g a.s./ha	**3.3**	**3.3**	**n.d.***	**n.d.***	**-**	**69.0**	**8.4**	**0.892 (n.s.)**
80 g a.s./ha	**0**	**0**	**29.5**	**- 28.1**	**0.435 (n.s.)**	**56.7**	**24.8**	**0.071 (n.s.)**
151 g a.s./ha	**6.7**	**6.7**	**24.6**	**- 7**	**0.967 (n.s.)**	**60.7**	**19.5**	**0.295 (n.s.)**
288 g a.s./ha	**0**	**0**	**16.6**	**27.8**	**0.265 (n.s.)**	**60.6**	**19.5**	**0.244 (n.s.)**
Reference	**80**	**80**	**n.d.**	**n.d.**	**n.d.**	**70.3**	**6.6**	**-**
LR_{50}				**> 288 g a.s./ha**				

n.s. not significant n.d. not detected, n.d. not detected due to capacity reasons
* one-way ANOVA, p-values are adjusted according to Dunnett

B. Observations

In the dose rates of 80 and 288 g a.s./ha 0% of mortality was observed. At the rates of 22 and 42 g a.s./ha 3.3% mortality were detected and 6.7% in the 151g a.s./ha rate. There was no reduction in reproductive success relative to the control at the 288; 151 and 80 g a.s./ha rates. No dose related repellent effect of the test item was observed.

CONCLUSION

The LR_{50} was estimated to be > 288 g a.s./ha.

IIA 8.8.2.2 Predatory mites

Report:	**KIIA 8.8.2.2/01, [redacted]; 2005**
Title:	Toxicity to the predatory mite *Typhlodromus pyri* SCHEUTEN (Acari, Phytoseiidae) using an extended laboratory test BYI 08330150 OD. Date: 2005-08-25
Organisation:	Bayer CropScience GmbH, Frankfurt, Germany
Report No.:	CW05/030; M-256306-01-1
Publication:	Unpublished
Dates of experimental work:	June 14, 2005 – June 28, 2005
Guidelines:	IOBC (Blümel et al. 2000)
Deviations:	very short declines in rel. humidity due to opening of the door
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the lethal and sublethal toxicity of freshly dried residues applied onto leaves of *Phaseolus vulgaris* var. *nanus*, to the predatory mite *Typhlodromus pyri*. The test item was applied at rates of 0.15; 0.51; 1.7; 5.9 and 20 g a.s./ha and the effects were compared to a toxic reference (a.s.: dimethoate) applied at 39.5 g a.s./ha, and a water treated control.

Mortality of 80 protonymphs was assessed 1, 3, 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 over the period of 7 - 14 days after treatment by counting the total number of offspring (eggs and larvae) produced.

In the highest dose rate of 20 g a.s./ha there was 100% corr. mortality. At the lower rates of 5.9; 1.7; 0.51 and 0.15 g a.s./ha Spirotetramat OD 150 98.6; 56.8; 1.4 and 4.1% corr. mortality were found. The reduction of reproduction at the rates of 0.51 and 0.15 g a.s./ha was 48.7 and 43.2%. The LR_{50} was calculated to be 1.588 g a.s./ha.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) 150 OD**
	Product code: AE 1302943 00 OD15 A101
Description	**Light brown granule**
Lot/batch No.	**Batch no.: 08030/0189(0152)**
	Tox no.: TOX07034-00
Analytical content	**148.89 g a.s./L**
Stability of test compound	**The stability of the test item and the reference item is guaranteed in the certificate of analysis.**
2. Vehicle and/or positive control	**Deionised water**
	Dimethoate: 392.1 g/L = 36.82% w/w
3. Test animals	
Species	**Predatory mite *Typhlodromus pyri***
Age	**Protonymphs**

Source	**Eggs of *Typhlodromus pyri* were supplied by [redacted]**
Acclimation period	**The test animals were kept under conditions recommended by the supplier until the start of the test.**
Environmental conditions	
Temperature	**24 - 25°C**
Photoperiod	**16:8 hours , 968 - 1026 lux**

B Study design and methods

1. In life dates **June 14, 2005 – June 28, 2005**

2. Experimental treatments

One exposure unit consisted of an intact leaf of *Phaseolus vulgaris*, which was laid after application on a layer of wet filter paper on top of a water soaked foam. A circle of insect glue (approx. 40 mm) was formed on the leaves. Sets of such cylinders were placed on a plastic tray such that the filter paper was constantly provided with deionised water.

The test concentrations and the suspension for the reference item were prepared stepwise on the day of application. Deionised water was used as diluent for both items. The items were applied to *Phaseolus vulgaris* leaves in the equivalent of 200 L/ha using a sprayer.

In total, 7 treatments were established (control, reference item, test item with 5 rates). Each treatment was replicated 4 times with 20 protonymphs per replicate. After the test units were set up (day 0) the protonymphs were placed onto the plant surface. The mites were transferred with a fine brush under a stereomicroscope and immediately afterwards examined to ensure they were undamaged and in good health. Then pollen (birch – pine mixture) was supplied as food and the units were kept under test conditions. The water supply for the mites was insured sticking a pin into each of the leaves. Additional food was added on days 3, 7 and 12.

3. Observations

On days 1, 3, 7, 10, 12 and 14 the number of dead and living mites was counted. Dead mites were removed. The number of escaped mites was calculated. Additionally on days 7, 10, 12 and 14 the number of females, males, eggs and juveniles was counted. Eggs and juveniles were removed.

At day +7 of each test, the number of dead and escaped mites was summed up for each replicate and calculated as percentages. A mean value of the four replicates was calculated.

The corrected mortality was obtained by comparing the values observed in the treated samples with those in the control samples, according to the formula of [redacted] (1947). The computer program SAS was used to perform the statistical analyses.

RESULTS AND DISCUSSION

A. Findings

The results are considered as valid since the mortality / escaping rate for the control stated in the laboratory method on glass was reached in this study (< 20% required; 7.5% in this study), the average mortality (dead and escaped mites = Mort/Esc-value) in the reference item was > 50% and the average number of eggs/female (calculated as sum of 4 assessment dates - from day 7 on) in the control group exceeded 4 eggs per female.

B. Observations

In this laboratory test the effects of BYI 08330 150 OD residues on the survival of the predatory mite *Typhlodromus pyri* were determined at the rates of 0.15; 0.51; 1.7; 5.9 and 20 g a.s./ha applied to bean leaves (*Phaseolus vulgaris*).

In the highest dose rate of 20 g a.s./ha there was 100% corr. mortality. At the lower rates of 5.9; 1.7; 0.51 and 0.15 g a.s./ha Spirotetramat OD 150 98.6; 56.8; 1.4 and 4.1% corr. mortality were found. The reduction of reproduction at the rates of 0.51 and 0.15 g a.s./ha was 48.7 and 43.2%.

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 in the table below:

Mortality and reproduction after exposure of *Typhlodromus pyri* to freshly dried residues of Spirotetramat OD 150 applied onto bean leaves

Treatment	Mortality				Reproduction		
	Uncorr.	Corr. [redacted]	P-value *	Rate	Rel. to control [%]	P-value **
Control	**7.5**	**-**		**7.3**	**-**	
0.15 g a.s./ha	**11.3**	**4.1**	**n.s.**	**4.1**	**43.2**	**0.081 (n.s.)**
0.51 g a.s./ha	**8.8**	**1.4**	**n.s.**	**3.7**	**48.7**	**0.060 (n.s.)**
1.7 g a.s./ha	**60.0**	**56.8**	**<0.0001 sign.**	**n.d.**		
5.9 g a.s./ha	**98.8**	**98.6**	**<0.0001 sign.**	**n.d.**		
20 g a.s./ha	**100**	**100**	**<0.0001 sign.**	**n.d.**		
Reference	**100**	**100**				
LR_{50} (95% CI)			**1.588 g a.s./ha (1.311 – 1.860)**			

n.s. not significant, sign. = significant, n.d. not detected

* Fisher's Exact test, two-sided, p-values are adjusted according to Bonferroni-Holm

** one-way ANOVA, p-values are adjusted according to Dunnett

CONCLUSION

The LR_{50} was calculated to be 1.588 g a.s./ha.

IIA 8.8.2.3 Ground dwelling predatory species

Covered by laboratory and extended laboratory studies on sensitive leaf-dwelling species: since spirotetramat will be applied to crops and not to bare ground, exposure of foliage-dwelling species can be considered to be more severe than in ground-dwelling species.

IIA 8.8.2.4 Foliage dwelling predatory species

Report: **KIIA 8.8.2.4/01, [redacted]; 2005**

Title: Dose-response toxicity (LR_{50}) of BYI 08330 150 OD to the green lacewing *Chrysoperla carnea* (STEPH.) under extended laboratory conditions (including food-application).

Date: 2005-12-14

Organisation:	[redacted], Germany Bayer CropScience GmbH, Frankfurt, Germany
Report No.:	051048082; M-262650-01-1
Publication:	Unpublished
Dates of experimental work:	August 18, 2005 - September 30, 2005
Guidelines:	IOBC(Vogt *et al.*, 2000), modified
Deviations:	modified for the extended laboratory test (exposure on natural substrate) in such a way that bean leaves were used instead of glass plates and that treated food was given
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the toxicity of freshly dried residues applied onto bean leaves to larvae of the green lacewing *Chrysoperla carnea* (Steph.). The test item was tested under extended laboratory conditions after exposure of larvae of *Chrysoperla carnea* to spray residues with rates of 44, 72, 112, 184 and 288 g a.s./ha in 200 L deionised water/ha applied on bean leaves and fed with *Sitotroga cerealella* eggs, which were treated at the same test item application rates as applied on the bean leaves. Survival of the larvae and pupae (pre-imaginal mortality) was used as primary test endpoint, with the aim to calculate the LR_{50}, if possible. Additionally, effects on fecundity were investigated. Behavioural impacts were recorded. The number of surviving larvae and hatched adults, as well as the number of eggs laid and larvae hatched (F1) were recorded over a period of 43 days.

No or only low effects on mortality were observed in all test item treatment groups. Therefore, a calculation of the LR_{50} (median lethal rate) was not possible. The LR_{50} is empirically estimated to exceed the highest tested application rate, i.e. 288 g a.s./ha. The reproductive output was considered as not impacted by the treatment.

Material and Methods

A Materials

1. Test material

Description	**Light brown suspension**
Lot/batch No.	**Batch no.: 08030/0189(0152)** **Tox no.: TOX07034-00**
Analytical content	**148.89 g a.s./L**
Stability of test compound	**The homogeneity was guaranteed by thorough shaking immediately before application. The test item was diluted under conditions corresponding to those in the field and applied on detached bean leaves and *Sitotroga cerealella* eggs. The stability under test conditions was therefore of no relevance for this type of experiment and was therefore not reported.**

2. Vehicle and/or positive control

Deionised water
Dimethoate EC 400 (30 mL product/ha in 200L/ha of water)

3. Test animals

Species	**Larvae of the green lacewing *Chrysoperla carnea* (Steph.), Neuropotera: Chrysopidae**
Age	**2-3 days**
Source	**[redacted] GmbH, D[redacted]; reared in the laboratory of the test facility since 2002**
Acclimation period	**reared in the laboratory of the test facility**
Environmental conditions	
Temperature	**23-26°C**
Photoperiod	**16:8 hours ; 2100 lux**

B Study design and methods

1. In life dates **August 18, 2005 - September 30, 2005**

2. Experimental treatments

The test consisted of two phases. In the exposure phase of the test, mortality of the larvae and pupae and adult emergence was determined following exposure to dried spray deposits on bean leaves and feeding with treated *Sitotroga* eggs. In the reproduction phase, the sublethal effects on reproductive performance (egg laying and hatching success) were assessed. In the exposure phase, 40 replicates each containing 1 larva
(2-3 days old) were established for each treatment group. In the reproduction phase of the test, all hatched adults were set up for each treatment group.
The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (30 mL product/ha in 200 L/ha of water) was used as a toxic reference treatment.
Exposure cages consisted of glass cylinders with gauze cover and a treated bean leaf on moistened filter paper as bottom. Cages were fixed to a glass plate and an acrylic plate.
Oviposition cages consisted of glass beakers covered with cotton gauze during egg laying. Hatching cages for F1 larvae consisted of plastic cages (Bellaplast) with clear cover.
Test plants were kidney beans (*Phaseolus vulgaris*, variety "Jutta"), grown in the laboratory of the test facility under controlled conditions. Primary leaves were cut shortly before the application, when the plants were 3 weeks old.
Larvae were fed with treated eggs of *Sitotroga cerealella* 3 times per week, adults with artificial food (diet according to the guideline) at each assessment day.
During the assessments the larvae were fed with treated (test item and control groups) and untreated (reference group) UV-sterilized eggs of *Sitotroga cerealella*.

3. Observations

The number of surviving larvae and hatched adults, as well as the number of eggs laid and larvae hatched (F1) were recorded over a period of 43 days. From these data the endpoints mortality and fecundity were calculated. The corrected mortality in the treatment groups was calculated according to ABBOTT (1925). For statistical calculation of mortality the software ToxRat Professional, Version 2.09 (RATTE, 2004) was used.

RESULTS AND DISCUSSION

A. Findings

All validity criteria according to Vogt *et al.* (2000) for conducting the laboratory test with *Chrysoperla carnea* and adapted to the extended laboratory test were met, since the mortality was 5%, the number of eggs per female and day was 18.9 and the mean hatching rate was 81% in the control. The mortality in the reference item group was 65% (corrected mortality: 63.2%).

Test item	Spirotetramat OD 150		
Test object	*Chrysoperla carnea* (Steph.)		
Exposure	Dried spray deposits on bean leaves and *Sitotroga* eggs		
Treatment	Mortality after 24 days [%]	Reproduction	
		Fecundity [mean number of eggs/ female]	Fertility mean hatching rate [%]
Control	5.0	18.9	81
Application rate [g a.s./ha]	Corrected mortality [%]		
44	5.3	19.5	80
72	7.9	18.7	81
112	2.6	20.2	81
184	0	18.6	80
288	5.3	19.0	80
Reference item	63.2	-	-
LR_{50}	> 288 g a.s./ha		

Statistical analysis (Fisher`s Exact Binomial-test, 1-sided, $p \leq 0.05$) revealed no significant differences concerning the mortality after 24 days between the control and all test item treatment groups.

B. Observations

No or only low effects on mortality were observed in all test item treatment groups. Therefore, a calculation of the LR_{50} (median lethal rate) was not possible. No significant effects were observed concerning reproduction. Fecundity was reduced by 1.6% in the 184 g and by -0.5% in the 288 g a.s./ha treatment compared to the control.

The reproductive output (mean number of eggs/female/day) was above the lower limit given as validity criterion for the glass plate method (mean fecundity of ≥ 15 eggs/female/day after 2 weeks) according to the historical database of the ring testing group (VOGT et al. 2000). According to that, this parameter was considered as not impacted by the treatment.

CONCLUSION

The LR_{50} is empirically estimated to exceed the highest tested application rate, i.e. 288 g a.s./ha.

Report:	**KIIA 8.8.2.4/02, [redacted]; 2006**
Title:	Toxicity to the ladybird beetle *Coccinella septempunctata* L. (Coleoptera, Coccinellidae) using an extended laboratory test including exposure to and oral uptake of BYI 08330 150 OD. Date: 2006-01-18
Organisation:	Bayer CropScience GmbH, Frankfurt, Germany
Report No.:	CW05/053; M-264175-01-1
Publication:	Unpublished
Dates of experimental work:	July 06, 2005 – August 24, 2005
Guidelines:	IOBC (Schmuck et al. 2000), modified
Deviations:	Modified for the extended laboratory test in such a way that bean leaves were used for exposure instead of glass plates; in addition, the larvae got treated food (aphids) for 4 days of the study to include a possible oral uptake of the test item and afterwards untreated food was given; on day 7 aphids on new plants were sprayed and this food was offered to larvae which had not yet pupated
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine the toxicity of freshly dried residues applied onto leaves of *Phaseolus vulgaris* to the ladybird beetle *Coccinella septempunctata*. The test item was applied to the upper surface of detached bean leaves at rates of 33; 57; 97; 168 and 288 g a.s./ha and the effects were compared to a toxic reference (a.s.: dimethoate) applied at 80mL product/ha, and a water treated control. The larvae were supplied *ad libitum* with aphids (*Acyrthosiphon pisum*) which had been treated for four days and afterwards fed with untreated aphids. On day 7 after application new aphids on bean plants were sprayed and fed until pupation was reached.

Pre-imaginal mortality was monitored over the duration of the study. After hatching the adult beetles were fed with aphids and pollen. The fertility and fecundity of the surviving hatched adults were then evaluated over a period of 14 days. Reproduction was assessed for all dose rates.

None of the dose rates had a significant influence on preimaginal mortality. The mean number of fertile eggs per female and day for the control during the test period was 6.0. Reproductive performance is considered as not impacted by the test item rates. The LR_{50} is empirically estimated to exceed the highest tested application rate, i.e. 288 g a.s./ha.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) 150 OD (Product code: AE 1302943 00 OD15 A101)**
Description	**Not specified in the report**
Lot/batch No.	**Batch no.: 08030/0189(0152)** **Tox no.: TOX07034-00**
Analytical content	**148.89 g a.s./L**

Stability of test compound	**The stability of the test item BYI 08330 150 OD and the reference item is guaranteed in the certificate of analysis.**
2. Vehicle and/or positive control	**Tap water**
3. Test animals	
Species	**Ladybird beetle *Coccinella septempunctata***
Age	**Larvae, 4 days old**
Source	**Eggs were supplied by [redacted], Germany.**
Acclimation period	**The eggs were kept under test conditions until the start of the study. *Acyrtosiphon pisum* (as food) was reared at the testing facility.**
Environmental conditions	
Temperature	**23.6 – 26.6°C**
Photoperiod	**16:8 hours ,**
	1050 - 1760 lux

B Study design and methods

1. In life dates **July 06, 2005 – August 24, 2005**

2. Experimental treatments

Exposure units consisted of a detached leaf (taken from bean plants) of *Phaseolus vulgaris*, Vollenda, which was laid on a wet cotton wool pad in a petri dish (9 cm diameter) after application. A constant supply of deionised water from the bottom was assured. Per individual test unit one steel ring (about 3 cm diameter and 3 cm high) was placed on the leaf. The top inner edge of the rings was coated with a lubricant (Hostaflon) in order to prevent the larvae from escaping.

The suspensions for the test and the reference item were prepared on the day of application, using tap water as diluent. They were applied in the equivalent of 200 L and 400 L water/ha to the detached bean leaves and bean plants, respectively. Application was done using a specially constructed sprayer, permitting application on a defined area. Once dry, the steel rings were placed onto the leaves into the petri dishes and one larva was added to each test unit.

Additionally potted bean plants with aphids (*Acyrthosiphon pisum* on *Vicia faba*) were sprayed on the day of application with the corresponding rates of the test item. The aphids were brushed from the leaves and were used as food for 4 days. Afterwards untreated aphids were fed until day 7. On day 7 new food was sprayed and offered to those larvae which had not yet pupated. After 3 days the remaining larvae and the adults were fed with untreated food.

In total, 7 treatments were established (control, reference item, test item with 5 rates (33, 57, 97, 168 and 288 g a.s./ha). Each treatment was replicated 40 times, with 1 larva each.

3. Observations

All test units were assessed daily and the condition of the ladybird beetles recorded. 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. Once the larvae had pupated, the emerged beetles were transferred to plastic boxes at the day of their individual hatching.
After this feeding period all beetles were transferred to glass jars. Their sex was determined and fresh stems of bean plants with aphids and untreated pollen were introduced as and when required. Sheets of black paper were offered to the beetles for egg-laying, which was checked daily. Pieces of paper with freshly laid eggs were cut out of the paper and stored in petri dishes. Mortality of the beetles during the egg laying period was recorded and the sex of the dead animals was determined.

RESULTS AND DISCUSSION

A. Findings

The mortality value for the control in this study was slightly above that mentioned in the guideline for glass plate studies (32.5% uncorr. mortality instead of 30%). Considering the special design of this study, it can be considered as valid, as for this type of study no validity criteria have been defined.
The mean number of fertile eggs per female and day for the control during the test period was 6.0. Reproduction was assessed for all dose rates of BYI 08330 150 OD.
As recommended by the guideline (for glass plate studies) the reproductive performance parameter can only be evaluated qualitatively, due to the very high species-inherent variability in egg-laying performance. As all values for the three highest and the lowest dose rate were within the historical data base for control beetles (≥ 2 fertile eggs per female and day), this parameter is considered as not impacted by the test item rates.

Mortality and reproduction after exposure of *Coccinella septempunctata* to freshly dried residues of Spirotetramat OD 150 applied onto detached bean leaves:

		Mortality [%]		Reproduction	
Treatment	[g a.s./ha]	Uncorr.	[redacted]- (corr.)	Fertility (hatching rate) [%]	Fertile eggs per female and day
Control (tap water)	-	32.5	-	99.3	6.0
BYI 08330 150 OD	33	32.5	-14.8	88.9	3.8
BYI 08330 150 OD	57	17.5	-22.2	91.7	1.8
BYI 08330 150 OD	97	32.5	0	89.3	3.6
BYI 08330 150 OD	168	47.5	22.2	99.0	2.0
BYI 08330 150 OD	288	50.0	25.9	86.7	4.1
Reference item	80 mL prod./ha	100	100	n.d.*	n.d.*
LR_{50}		> 288 g a.s./ha			

* n.d.: not determined

B. Observations

The dose rates of 33; 57; 97; 168 and 288 g a.s./ha had no significant influence on preimaginal mortality. Concerning reproduction, no significant effects were observed. For the

highest treatment rates fertility was reduced by 0.3% at 168 g and by 12.7% at 288 g a.s./ha compared to the control.

CONCLUSION

The LR_{50} is empirically estimated to exceed the highest tested application rate, i.e. 288 g a.s./ha.

IIA 8.8.2.5 Other terrestrial invertebrates

Further studies with other terrestrial non target arthropods are not deemed necessary.

IIA 8.9 Effects on earthworms

IIA 8.9.1 Acute toxicity to earthworms

Report:	**KIIA 8.9.1/01, [redacted]; 2004**
Title:	BYI 08330 (tech.): Acute Toxicity to Earthworms (*Eisenia fetida*) tested in Artificial Soil with 5 % Peat. Date: 2004-01-07
Organisation:	[redacted], Germany
Report No.:	LKC/Rg-A-18/03; M-104105-01-2
Publication:	Unpublished
Dates of experimental work:	November 11, 2003 – November 26, 2003
Guidelines:	OECD 207, "OECD-Guideline for Testing Chemicals," "Earthworm, Acute Toxicity Tests" (1984)
Deviations:	yes With respect to the properties of the test item (log Pow < 2) 5% peat instead of 10% peat were used considering the influence on bioavailability (EPPO 2002)
GLP:	yes (certified laboratory)

Executive summary

The aim of this study was to assess the effect of BYI 08330 (tech.) on survival and growth of the earthworm *Eisenia fetida* during a 14 days exposure in an artificial soil at 6 different application rates.

For this purpose adult earthworms (14 x 10 animals per concentration, the overall average weight was 0.36 g) were exposed for 14 days in an artificial soil to the nominal concentrations of 32, 100, 178, 316, 562 and 1000 mg a.s./kg dry weight soil. The artificial soil comprised 5% peat. Physical-chemical measurements showed that the properties of the test soil are in agreement with the nominal values. No morphological and behavioural effects were observed.

The endpoints of the test were based on nominal concentrations, since an analytical check of the test concentrations for this 14 day acute test was not specified in the reference guideline.

Since no mortality was observed within the test, the LC_{50} (14 d) could not be calculated and was estimated to be > 1000 mg a.s./kg dry weight substrate. Related to weight alterations and symptoms, the no-observed-effect-concentration (NOEC) was 1000 mg a.s./kg dry weight soil, the lowest-observed-effect-concentration (LOEC) > 1000 mg a.s./kg dry weight soil.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**White powder**
Lot/batch No.	**Batch no.: MIX-Batch 08045/0014** **TOX no.: 6344-00**
Purity	**97.5%**
Stability of test compound	**Approved until 2003-23-12 when stored at room temperature**

2. Vehicle and/or positive control	**Quartz sand** **Under the same conditions a study (non-GLP) was carried out with the reference substance Chloroacetamide A.R. starting December 2, 2002. The nominal test concentrations were 5.6, 10, 18, 24 and 32 mg/kg.**
3. Test animals	
Species	***Eisenia fetida andrei***
Age	**Adult worms, > 2 months**
Source	**Strain of Prof [redacted], Germany held in the laboratory for several years**
Acclimation period	**On the day prior to the beginning of the study, the earthworms were transferred from the breeding substrate to an artificial soil (without test item) under the test conditions.**
Environmental conditions	
Temperature	**20 ± 2°C**
Photoperiod	**constant light, 400 – 800 lux**

B Study design and methods

1. In life dates **November 11, 2003 – November 26, 2003**

2. Experimental treatments

In order to cultivate the test organisms a breeding substrate was used, which consisted of about 70% natural soil, 25% peat and 5% straw (dry weight in each case). The animals were fed on ground, dried cattle manure at 14 day intervals. At the same time, the substrate was also replenished with water to bring the humidity about 70 - 90%. The animals were transferred into fresh substrate at half-yearly intervals. The temperature was 22 ± 2°C with a 12:12 hours light-dark cycle.

The artificial soil used in the test, was prepared according to the guideline. With respect to the properties of the ingredients of the test item (log $P_{OW} \geq 2$) 5% peat instead of 10% peat was used considering the influence on bioavailability (EPPO 2002). It was prepared with the following composition (percentage distribution on dry weight basis): Sphagnum peat (5%, pH 2 – 4), Kaolinite clay (20%, content of Kaolinite 56%), industrial quartz sand (74%, particle size of 0.2 – 0.05 mm: 68.2%) and Calcium carbonate (about 0.2 - 1%) for the adjustment to pH 6.0 ± 0.5.

The range of test concentrations used in this test was based on an acute pre-test (non GLP) with *Eisenia fetida* and the same test substance. To obtain the nominal test concentrations of 32, 100, 178, 316, 562 and 1000 mg a.s./kg dry weight soil, nominal amounts of 16, 50, 89, 158, 281 and 500 and 16 mg test item were weighed into a petri dish and quartz sand (washed and calcined) was added up to a final weight of 5 grams and then thoroughly mixed. For each of the four replicates and six concentrations the application mixture was prepared separately and then mixed

with artificial soil (500 g dry weight). To the control replicates 5 g of quartz sand (without test item) was mixed into the soil. Finally 50 mL of deionised water were added and mixed into each replicate to achieve between 40% and 60% of the maximum water holding capacity (WHC_{max}).

The test containers were 1.5 L preserving jars, covered with glass lids, filled with 500 g dry weight test soil (equivalent to 625 g wet weight). Ten earthworms were placed in each test container, for each test substance concentration and the control group four replicates were tested. The animals were not fed during the study.

3. Observations

Peat moisture was determined prior to preparation of the artificial soil, artificial soil moisture was determined prior to the start and at the end of the test (weight difference before and after drying). The maximum water holding capacity ($WHC_{max.}$) of the artificial soil was determined in 2 samples of the artificial soil. The pH was measured at the start and the end of the study (50 g of the test soil were resuspended in 100 mL of 0.1 mol/L KCl solution).

At the beginning (prior to exposure) and at the end of the 2 weeks of exposure, the adult test organisms of each vessel were weighed. At the start each worm was weighed individually, at the end worms from each test vessel were weighed together. Seven days after the start of the study, the number of surviving earthworms was determined by emptying the soil out onto an inert surface and removing the earthworms by hand. The animals were then returned to the test container with the test soil. After 14 days, the weight, abnormal behaviour, observed symptoms as well as the number of surviving earthworms were determined. Earthworms which showed no reaction when prodded with a blunt probe were considered dead.

The analytical concentration of the active substance was not determined, since an analytical check of the test concentrations for this 14 days acute test was not specified in the reference guideline.

The data on weight alteration of the test organisms after 2 weeks of exposure were statistically analysed with Dunnett´s multiple t-test (alpha = 0.05, one-sided smaller). Previous statistical testing of the data showed that they fitted the assumption of normal distribution (Kolmogorroff-Smirnov test) and showed homogeneity of variances as checked by Cochran´s test. The statistic software used was ToxRatPro Version 2.07.

RESULTS AND DISCUSSION

A. Findings

Validity criteria were all met as the mortality rate in the control was below 10% which can be regarded as the limit for natural mortality. As the physical-chemical measurements showed, the properties of the test soil are in agreement with the nominal values. The water content in the artificial soil was about 21% at the start and 19.6 - 20.1% at the end of the study, the water content of WHC_{max} was 59.8 to 60.5%, the pH ranged from 6.35 to 6.41.

The LC_{50} of the reference substance (14 days) was calculated to be 23 mg Chloroacetamide A.R./kg dry weight soil (95% Cl 21 - 24 mg Chloroacetamide A.R./kg) that is within the usual range determined in international ring studies.

Effects of BYI 08330 (tech.) on *Eisenia fetida* after 14 days exposure (Mortality (%) was calculated from the means of 4 replicates each containing ten earthworms ± standard deviation).

Test concentrations [mg a.s./kg dry weight soil]	Mortality [%]	Weight alteration of the survivors [%]	Dunnett-Test
control	0	- 9 ± 2	
32	0	- 9 ± 2	n.s.
100	0	- 8 ± 2	n.s.
178	0	- 9 ± 4	n.s.
316	0	- 7 ± 2	n.s.
562	0	- 7 ± 2	n.s.
1000	3 ± 5	- 8 ± 2	n.s.

n.s. = not significant (weights of control and the test concentration do not differ statistically significantly in Dunnett Test)

Toxicity to earthworms after 14 days

Test item	**Spirotetramat (tech.)**
Test object	***Eisenia fetida***
Exposure	**14 d**
LC_{50}	**> 1000 mg/kg dry weight soil**
NOEC (no-observed-effect-concentration)	**≥ 1000 mg/kg dry weight soil**
LOEC (lowest-observed-effect-concentration)	**> 1000 mg/kg dry weight soil**

B. Observations

No morphological and behavioural effects were observed.

CONCLUSION

Since only 3% mortality was observed for the highest concentration tested, the LC_{50} (14 d) could not be calculated and was estimated to be > 1000 mg a.s./kg dry weight substrate. Related to weight alterations and symptoms, the no-observed-effect-concentration (NOEC) was 1000 mg a.s./kg dry weight soil, the lowest-observed-effect-concentration (LOEC) > 1000 mg a.s./kg dry weight soil.

Acute effects of metabolites on earthworms

No acute test has been conducted with BYI 08330-enol as a chronic study is available wherein an acute LC_{50} was determined (see KIIA 8.9.2/01).

Report:	**KIIA 8.9.1/02, [redacted]; 2005**
Title:	BYI 08330-cis-ketohydroxy: Acute Toxicity to Earthworms (*Eisenia fetida*) tested in Artificial Soil. Date: 2005-10-17
Organisation:	[redacted], Germany
Report No.:	LKC/Rg-A-47/05, M-258925-01-2
Publication:	Unpublished
Dates of experimental work:	February 9, 2005 - March 2, 2005
Guidelines:	OECD 207, "OECD-Guideline for Testing Chemicals," "Earthworm, Acute Toxicity Tests" (1984)
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The aim of this study was to assess the effect of BYI 08330-cis-ketohydroxy on survival and growth of the earthworm *Eisenia fetida* during a 14 days exposure in an artificial soil at 5 application rates.

For this purpose adult earthworms (4 x 10 animals per concentration, the overall average weight was 0.34 g) were exposed for 14 days in an artificial soil to the nominal concentrations of 10, 32, 100, 316 and 1000 mg p.m./kg dry weight soil. The artificial soil comprised 10% peat. Physical-chemical measurements showed that the properties of the test soil are in agreement with the nominal values. No morphological and behavioural effects were observed.

Since no mortality was observed within the test, the LC_{50} (14 d) was > 1000 mg p.m./kg dry weight soil. Related to weight alterations, the no-observed-effect-concentration (NOEC) was 1000 mg p.m./kg dry weight soil, the lowest-observed-effect-concentration (LOEC) was > 1000 mg p.m./kg dry weight soil. The values given for concentrations are nominal concentrations.

MATERIAL AND METHODS

A Materials

1. Test material	**BYI 08330-cis-ketohydroxy**
Description	**Light yellow solid**
Lot/batch No.	**Batch no.: NLL7549-7** **TOX no.: 06867-00**
Purity	**98.6%**
Stability of test compound	**Approved until 2005-07-27 when stored at room temperature**
2. Vehicle and/or positive control	**Quartz sand** **Under the same conditions a study (non-GLP) was carried out with the reference substance Chloroacetamide A.R. starting November 2,**

	2004. The nominal test concentrations were 5.6, 10, 18, 24 and 32 mg/kg.
3. Test animals	
Species	*Eisenia fetida andrei*
Age	Adult worms, > 2 months
Source	Strain of Prof [redacted], Germany; held in the laboratory for several years
Acclimation period	On the day prior to the beginning of the study, the earthworms were transferred from the breeding substrate to an artificial soil (without test item) under the test conditions.
Environmental conditions	
Temperature	20 ± 2°C
Photoperiod	constant light, 400 – 800 lux

B Study design and methods

1. In life dates February 9, 2005 - March 2, 2005

2. Experimental treatments

In order to cultivate the test organisms a breeding substrate was used, which consisted of about 70% by weight of natural soil, 25% peat and 5% straw (dry weight in each case). The animals were fed on ground, dried cattle manure at 14 day intervals. At the same time, the substrate was also replenished with water. The animals were transferred into fresh substrate at half-yearly intervals. The temperature was 20 ± 2°C with a 12 : 12 hours light-dark cycle.

The artificial test soil was prepared with the following composition (percentage distribution on dry weight basis): Sphagnum peat (10%, pH 2 – 4), Kaolinite clay (20%, content of Kaolinite 56%) and industrial quartz sand (70%, particle size 0.20 mm – 0.05 mm: 68.2%). The pH value was in the range of 6.0 ± 0.5, so no adjustment with calcium carbonate was necessary.

Five concentrations and a control were tested. To obtain the nominal test concentrations of 1000, 316, 100 and 32 mg test item/kg dry weight soil, nominal amounts of 500, 158, 50 and 16 mg test item were weighed into a petri dish respectively and quartz sand (washed and calcined) was added up to a final weight of 5 grams and then thoroughly mixed (test concentration of 10 mg test item/kg dry weight soil: 20.0 mg test item was added up to 20 g). Each application mixture (5 g) was transferred separately to artificial soil (500 g dry weight) and mixed thoroughly. To the control replicates 5 g of quartz sand (without test item) was mixed into the soil. Finally 50 mL of deionized water were added and mixed into each replicate to achieve between 40% and 60% of the maximum water holding capacity (WHC_{max}).

The test containers were 1.5 litre preserving jars, covered with glass lids, filled with 500 g dry weight test soil (equivalent to 625 g wet weight). Ten earthworms were

placed in each test container, for each test substance concentration and the control group four replicates were tested. The animals were not fed during the study.
The analytical concentration of the pure metabolite was not determined, since an analytical check of the test concentrations for this 14 days acute test was not specified in the reference guideline.

3. Observations

Peat moisture was determined prior to preparation of the artificial soil, artificial soil moisture was determined prior to the start and at the end of the test (weight difference before and after drying). The maximum water holding capacity ($WHC_{max.}$) of the artificial soil was determined in 2 samples of the artificial soil. The pH was measured at the start and the end of the study (50 g of the test soil were resuspended in 100 mL of 0.1 mol/L KCl solution), the light intensity was measured on day 0, 7 and 14.
At the beginning (prior to exposure) and at the end of the 2 weeks of exposure, the adult test organisms of each vessel were weighed. At the start each worm was weighed individually, at the end worms from each test vessel were weighed together. Seven days after the start of the study, the number of surviving earthworms was determined by emptying the soil out onto an inert surface and removing the earthworms by hand. The animals were then returned to the test container with the test soil. After 14 days, the weight, abnormal behaviour, observed symptoms as well as the number of surviving earthworms were determined. Earthworms which showed no reaction when prodded with a blunt probe were considered dead.
The data on weight alteration of the test organisms after 2 weeks of exposure were statistically analysed with Dunnett´s multiple t-test (alpha = 0.05, one-sided smaller). Previous statistical testing of the data showed that they fitted the assumption of normal distribution (Kolmogorroff-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. Findings

Validity criteria were all met as the mortality rate in the control was below 10% which can be regarded as the limit for natural mortality. As the physical-chemical measurements showed, the properties of the test soil were in agreement with the nominal values. The water content in the artificial soil was about 26.5% at the start and 24.5 – 24.9% at the end of the study, the average water content of WHC_{max} was 60.3%, the pH ranged from 6.19 to 6.46 and the measured mean light intensity ranged from 557 - 625 lux.
The LC_{50} of the reference substance (14 days) was calculated to be 9.3 mg Chloroacetamide A.R./kg dry weight soil that is within the usual range determined in international ring studies. The 95% confidence limits were not determined due to mathematical reasons.

Effects of BYI 08330-cis-ketohydroxy on *Eisenia fetida* after 14 days exposure (Mortality (%) was calculated from the means of 4 replicates each containing ten earthworms ± standard deviation).

Test concentrations [mg p.m./kg dry weight soil]	Mortality [%]		Weight alteration of the survivors	
	7 days	14 days	[%]	Dunnett-Test
control	0	0	+ 2 ± 1	
10	0	0	+ 3 ± 3	n.s.
32	0	0	+ 3 ± 3	n.s.
100	0	0	+ 2 ± 1	n.s.
316	0	0	+ 12 ± 1	n.s.
1000	0	0	+ 2 ± 1	n.s.

n.s. = not significant (weights of control and the test concentration do not differ statistically significantly in Dunnett Test, α = 0.05, one-sided smaller)

Toxicity to earthworms after 14 days

Test item	**BYI 08330-cis-ketohydroxy**
Test object	***Eisenia fetida***
Exposure	**14 d**
LC_{50}	**> 1000 mg/kg dry weight soil**
NOEC (no-observed-effect-concentration)	**≥ 1000 mg/kg dry weight soil**
LOEC (lowest-observed-effect-concentration)	**> 1000 mg/kg dry weight soil**

B. Observations

No morphological and behavioural effects were observed.

CONCLUSION

Since no mortality was observed within the test, the LC_{50} (14 d) was estimated to be > 1000 mg p.m./kg dry weight soil. Related to weight alterations and symptoms, the no-observed-effect-concentration (NOEC) was ≥ 1000 mg p.m./kg dry weight soil, the lowest-observed-effect-concentration (LOEC) > 1000 mg p.m./kg dry weight soil.

Report:	**KIIA 8.9.1/03, [redacted]; 2008**
Title:	Spirotetramat - 4-methoxycyclohexanon: Acute toxicity to earthworms (*Eisenia fetida*) tested in artificial soil with 5% peat. Date: 2008-01-09
Organisation:	[redacted], Germany
Report No.:	LRT-Rg-A-98/07; M-296072-01-1
Publication:	unpublished
Dates of experimental work:	November 28, 2007 - December 13, 2007
Guidelines:	OECD-Guideline No. 207 "OECD-Guideline for Testing of Chemicals," "Earthworm, Acute Toxicity Tests", April 4, 1984
Deviations:	Deviations: Yes • With respect to the properties of the test item (LogPow = 2) 5% peat instead of 10% peat were used considering the influence on bioavailability (EPPO 2003). • At the start of the study, the individual body weight of some of the earthworms was lower than 300 mg, as recommended in the guideline.
GLP:	yes (certified laboratory)

Executive summary

The aim of this study was to assess the effect of Spirotetramat-4-methoxycyclohexanon on survival and growth of the earthworm *Eisenia fetida* during a 14 days exposure in an artificial soil at 5 application rates.

For this purpose adult earthworms (4 x 10 animals per concentration, the overall average weight was 0.29 g) were exposed for 14 days in an artificial soil to the nominal concentrations of 62.5, 125, 250, 500 and 1000 mg test item / kg dry weight soil. The artificial soil comprised 5% peat. Physical-chemical measurements showed that the properties of the test soil are in agreement with the nominal values. No morphological and behavioural effects were observed.

In this study the LC_{50} (14 d) of Spirotetramat - 4-methoxycyclohexanon to earthworms (*Eisenia fetida*) was determined to be greater than 1000 mg test item/kg dry weight soil. Based on the effects of body weight, the Lowest Observed Effect Concentration (LOEC) of Spirotetramat - 4-methoxycyclohexanon to earthworms (*Eisenia fetida*) was determined to be 250 mg test item/kg dry weight soil. The No Observed Effect Concentration (NOEC) was determined to be 125 mg test item/kg dry weight soil.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat-4-methoxycyclohexanon**
Description	**Colourless liquid**
Lot/batch No.	**Batch code: AE 1302938-01-01;** **Origin Batch No.: SAV 5506-001;** **TOX-No.: 08108-00**
Purity	**97.9%**
Stability of test compound	**Approved until 2008-05-20 when stored at +25 ±5°C**
2. Vehicle and/or positive control	**Quartz sand** **Under the same conditions a study (non-GLP) was**

	carried out with the reference substance Chloroacetamide A.R. from October to November 2, 2006. The nominal test concentrations were 5.6, 10, 18, 24 and 32 mg/kg.
3. Test animals	
Species	*Eisenia fetida andrei*
Age	Adult worms, > 2 months
Source	Strain of Prof [redacted] Germany; held in the laboratory for several years
Acclimation period	On the day prior to the beginning of the study, the earthworms were transferred from the breeding substrate to an artificial soil (without test item) under the test conditions.
Environmental conditions	
Temperature	20 ± 2°C
Photoperiod	constant light, 400 – 800 lux

B Study design and methods

1. In life dates November 28, 2007 - December 13, 2007

2. Experimental treatments

The strain has been held in the laboratory for several years under the conditions described in the BBA Proposal for an OECD Guideline (ECO 85, = UPEC 15, February 1981): 20 +2°C, 12:12 hours light-dark cycle. The breeding substrate consists of about 70% by weight of natural soil, 25% peat and 5% straw (dry weight in each case). The animals are fed on ground, dried cattle manure at 14 day intervals. At the same time, the substrate is also replenished with water. The animals are transferred into fresh substrate at half-yearly intervals.

The artificial soil was prepared according to the guideline. With respect to the properties of the ingredients of the test item (Log $P_{ow} \geq 2$) 5% peat instead of 10% peat was used considering the influence on bioavailability (EPPO 2003).

The artificial soil was prepared with the following composition (percentage distribution on dry weight basis): Sphagnum peat: 5% (air dried and finely ground, pH 2 - 4), Kaolinite clay: 20% (content of Kaolinite ($Al_2Si_2O_5(OH)_4$ = 56%), Industrial quartz sand (Sort: F 36): 74.8% (Particle size: 0.20 mm - 0.05 mm = 73.0%) and Calcium carbonate ($CaCO_3$): 0.2% (for the adjustment to pH 6.0 ± 0.5).

Five concentrations and a control were tested. To obtain the nominal test concentrations of 1000, 500, 250, 125 and 62.5 mg test item/kg dry weight soil, a sufficient amount of the application mixtures was prepared separately for all test concentrations. Uniform masses of the application mixtures was used for all test concentrations. They were thoroughly mixed into the artificial soil of each replicate using a laboratory mixer in the order lowest application rate to highest application rate. Finally 50 mL of deionised water were added and mixed into each replicate to achieve soil moisture of between 40 and 60% of the

maximum water holding capacity (WHC_{max}). All application mixtures were prepared freshly on the day of application.
The control group was treated first in the same way as described above but with untreated quartz sand only.
The test containers were 1.5 litre preserving jars, covered with glass lids. For each test container, 500 g dry weight test soil (equivalent to 580 g wet weight) was prepared. For each test item concentration and the control group, four replicates of ten earthworms (4 x 10) were tested. The animals were not fed during the study.
The analytical concentration of the pure metabolite was not determined, since an analytical check of the test concentrations for this 14 days acute test was not specified in the reference guideline.

3. Observations

Peat moisture was determined prior to preparation of the artificial soil. Artificial soil moisture was determined directly after test start (day 0) and at the end of the test.
The maximum water holding capacity (WHC max) of the artificial soil was determined in 2 samples of the artificial soil: the soil was filled into a cylindrical vessel made of 2Asteel (diameter 6 cm, height 7.8 cm).
The pH was measured at the start and the end of the study (50 g of the test soil were resuspended in 100 mL of 0.1 mol/L KCl solution), the light intensity was measured on day 0, 7 and 14.
At the beginning (prior to exposure) and at the end of the 2 weeks of exposure, the adult test organisms of each vessel were weighed. At the start each worm was weighed individually, at the end worms from each test vessel were weighed together.
Seven days after the start of the study, the number of surviving earthworms was determined by spreading the soil onto an inert surface and removing the earthworms by hand. The surviving animals were then returned to the test container with the test soil. After 14 days, the weight, abnormal behaviour observed symptoms as well as the number of surviving earthworms were determined. Earthworms which showed no reaction when prodded with a blunt probe were considered dead.
The data on weight alteration of the test organisms after 2 weeks of exposure were statistically analysed with Dunnett's multiple t-test (alpha = 0.05, one-sided smaller). Previous statistical testing of the data showed that they fitted the assumption of normal distribution (Kolmogorroff-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. Findings

The mortality rate in the control was below 10% which can be regarded as the limit for natural mortality. As the physical-chemical measurements show, the properties of the test soil are in agreement with the nominal values. The LC_{50} of the reference substance is within the usual range. The test conditions are therefore equivalent to the standard. The water content in the artificial soil was about 20.7% at the start and 20.3 – 20.5% at the end of the study, the

average water content of WHC_{max} was 60.9 – 61.6%, the pH ranged from 5.52 to 5.78 and the measured mean light intensity ranged from 592 - 619 lux.
The LC_{50} of the reference substance (14 days) was calculated to be 11.1 mg Chloroacetamide A.R./kg dry weight soil that is within the usual range determined in international ring studies.
The 95% confidence limits were 10.1 - 12.2 mg Chloroacetamide A.R./kg dry weight soil.

Effects of Spirotetramat - 4-methoxycyclohexanon on *Eisenia fetida* after 14 days exposure

Test concentrations (a) [mg test item/kg dry weight soil]	Mortality [%](b) after		Weight alteration of the survivors	
	7 days	14 days	[%](b)	Dunnett-Test(c)
control	0	0	+ 8.93 ± 6.07	
62.5	0	0	+ 3.19 ± 2.32	n.s.
125	0	0	+ 2.51 ± 3.14	n.s.
250	0	0	- 4.24 ± 2.32	s.
500	0	0	- 13.66 ± 5.36	s.
1000	0	0	- 20.88 ± 7.45	s.

Mortality (%) was calculated from the means of 4 replicates each containing ten earthworms.
s. = weights of control and the test concentration do differ statistically significantly
n.s. = weights of control and the test concentration do not differ statistically significantly
(a) test concentrations are nominal concentrations
(b) mean ± standard deviation
(c) Dunnett Test (alpha = 0.05, one-sided, smaller)

Toxicity to earthworms after 14 days

Test item	**Spirotetramat-4-methoxycyclohexanon**
Test object	***Eisenia fetida***
Exposure	**14 d**
LC_{50}	**> 1000 mg/kg dry weight soil**
NOEC (no-observed-effect-concentration)	**125 mg/kg dry weight soil**
LOEC (lowest-observed-effect-concentration)	**250 mg/kg dry weight soil**

B. Observations

No morphological and behavioural effects were observed.

CONCLUSION

In this study the LC_{50} (14 d) of Spirotetramat - 4-methoxycyclohexanon to earthworms (*Eisenia fetida*) was determined to be greater than 1000 mg test item/kg dry weight soil. Based on the effects of body weight, the Lowest Observed Effect Concentration (LOEC) of Spirotetramat - 4-methoxycyclo-hexanon to earthworms (*Eisenia fetida*) was determined to be 250 mg test item/kg dry weight soil. The No Observed Effect Concentration (NOEC) was determined to be 125 mg test item/kg dry weight soil.

IIA 8.9.2 Sublethal effects on earthworms

Chronic effects of metabolites on earthworms

Report:	**KIIA 8.9.2/01, [redacted] & [redacted]; 2005**
Title:	BYI 08330-Enol: Reproduction toxicity to the earthworm *Eisenia fetida* in artificial soil. Date: 2005-01-19
Organisation:	ECT Oekotoxikologie GmbH, Flörsheim, Germany [redacted], Germany
Report No.:	P18RR; M-243305-01-2
Publication:	Unpublished
Dates of experimental work:	November 09, 2004 – January 04, 2005
Guidelines:	ISO Guideline 11268-2 "Soil quality – Effects of pollutants on earthworms (*Eisenia fetida*) Part 2: Determination of effects on reproduction" adopted July 1998
Deviations:	No major deviations
GLP:	yes (certified laboratory)

Executive summary

The purpose of this study was to determine a NOEC/LOEC and an EC_{50} for the effects of BYI 08330-Enol on the reproduction (56 days after application), mortality and the biomass development (28 days after application) of the earthworm *Eisenia fetida* (Lumbricidae) by dermal and alimentary uptake using a standardised artificial soil.

The test item was mixed with the substrate. Ten earthworms per replicate (4) were exposed to the test item for 28 days at nominal concentrations of 10, 32, 100, 316 and 1000 mg pure metabolite/kg artificial dry weight soil (mg p.m./kg d.wt.s.). After 28 day of exposure, the adult worms were removed and the cocoons produced by these animals were kept for a further 28 days in the treated artificial soil. At the end of the test period (i.e. after 56 days) the juvenile worms hatched from these cocoons were extracted from the artificial soil. The artificial soil comprised 10% peat.

Physical-chemical measurements showed that the properties of the test soil are in sufficient agreement with the nominal values. With the exception of reduced ingestion no morphological and behavioural effects were observed during the test period.

0% mortality was observed in the control and 0 – 2.5% mortality at all concentrations of the test item. Thus, an LC_{50} value could not be calculated, but was regarded as > 1000 mg p.m./kg d.wt.s. The $NOEC_{Reproduction}$ was determined as 100 mg p.m./kg d.wt.s. The data corresponded to nominal concentrations.

MATERIAL AND METHODS

A Materials

1. Test material	**BYI 08330-Enol**
Description	**Beige powder**
Lot/batch No.	**Batch no.: 692-101-09-0005** **TOX no.: 06850-00**

Purity	**99.1%**
Stability of test compound	**Stable until 2005-3-17, when stored dry, at temperatures < 40°C**
2. Vehicle and/or positive control	**Quartz sand** **Carbendazim as the active ingredient of the fungicide Derosal was tested as the toxic reference item from August 11 - October 06, 2004. It was used in concentrations of 1, 3 and 5 mg a.s./kg.**
3. Test animals	
Species	***Eisenia fetida***
Age	**Adult worms (with clitellum), between 2 months and 1 year old and with a fresh weight between 300 and 600 mg**
Source	**Originally delivered by Co. Landenberger (D-72355 Schömberg), since February 1994 kept at the ETC**
Acclimation period	**On the day prior to the beginning of the study, the earthworms were transferred from the breeding substrate of a synchronised culture to an artificial soil (without test item) under test conditions.**
Environmental conditions	
Temperature	**20 ± 2°C**
Photoperiod	**16:8 light-dark cycle, 400 - 800 lux**

B Study design and methods

1. In life dates **November 9, 2004 - January 4, 2005**

2. Experimental treatments

Breeding and keeping of the test organisms was described in detail elsewhere (Standard Operation Procedures (SOP) A 7.1). The synchronised breeding culture was initiated approx. 10 months before starting the test.

The artificial test soil was prepared with the following composition (percentage distribution on dry weight basis): Sphagnum peat (10%), Kaolin clay (20%, content of Kaolinite > 30%), industrial quartz sand (69.55%, particle size 0.20 mm – 0.05 mm; > 50%) and 0.45% Calcium carbonate for the adjustment to pH 6.0 ± 0.5. Also dried food material (5 g finely ground cow manure /500 g d.wt.) was mixed into the artificial soil. After intensely mixing the soil was wetted with deionised water to reach a water content of 40 - 60% of the maximum water holding capacity (WHC_{max}). The water content of the test substrate was checked weekly by reweighing the test vessels and compensating the loss of water.

BYI 08330-Enol was mixed thoroughly in an amount of quartz sand sufficient to prepare a mixture for each test item concentration separately. An appropriate

amount of these test item/quartz sand mixtures served to prepare the different concentrations of the test item in the artificial soil. 500 g d.wt. of the treated artificial soil was filled into the test vessels, so the test item was applied once at the beginning of the test. Five concentrations (10, 32, 100, 316 and 100 mg p.m./kg d.wt.s.) and a control were tested.

Test vessels were Bellaplast containers with an area of 11 x 15.5 cm and a height of 6 cm. The test vessels were covered by a transparent cover. Ten earthworms were placed in each test container, for each test substance concentration and the control group four replicates were tested. The adult worms were fed with 5 g food per test vessel weekly. If the worms did not feed, the amount of food was reduced to minimise the risk of moulding.

The analytical concentration of the pure metabolite was not determined, since an analytical check of the test concentrations is not provided according to ISO Guideline 11268-2.

3. Observations

Moistening was observed on day 1, 7, 14, 21, 28, 35, 42 and 49 by determination of the weight of the test containers and compensating the water losses by addition of deionised water. At the start and the end of the study the moisture (weight difference before and after drying) and the maximum water holding capacity ($WHC_{max.}$) of the artificial soil were determined. The pH was measured at the start and the end of the study as well.

The feeding activity was recorded for each test container on day 1, 7, 14, 21 and 28. On day 28 the adult worms were removed from the substrate. During this process morphological and behavioural changes and the number of surviving worms in each test container were recorded. Worms were classified as dead when they did not respond to a gentle mechanical stimulus to the front end. Individual weighing of the surviving worms. The artificial soil (including cocoons) was put back into the test containers and the test continued for another 28 days.

On day 56 the juvenile worms were sampled out of the test containers. Extraction was done using the water bath method (50 - 60°C water temperature), followed by hand-sorting in one replicate per treatment to control the extraction efficiency. During this process morphological and behavioural changes and the number of juveniles in each test container were recorded.

No statistical analysis was performed concerning mortality. For biomass and reproduction data were checked for normality by R/s test procedure and for homogeneity by Cochran's test. Treatment means were compared by ANOVA followed by Dunnett's t-test and Williams t-test respectively ($p \leq 0.05$, according to the guideline two-sided for biomass and one-sided for reproduction) and tested for statistically significant differences compared to the control. The statistical software package ToxRat Professional 2.09 was used for these calculations.

RESULTS AND DISCUSSION

A. Findings

The validity criteria according to the guideline were met as mortality of the adult test animals in the control was ≤ 10% (being 0%), the mean number of juveniles per replicate in the control was ≥ 30 (being 148) and the coefficient of variation for the number of juveniles in the control was ≤ 30% (being 29.3%).
The temperature ranged from 17 – 22°C (for few and short time periods below 18°C, however, this is considered not to influence the result) by a light intensity of 485 - 558 lux, the pH values ranged from 6.1 – 6.2 at the start and from 6.8 – 7.0 at the end of the test and the soil moisture was in the range of 28.9 – 35.4% of the dry weight of the artificial soil. For all concentrations of the test item tested the pH-value was 0.3 – 0.5 units higher than required by the guideline at the end of the test. However, changes in the pH-value by approx. 0.5 - 1 units can be often observed in tests with earthworms. The soil moisture was 0.7% of WHC_{max} higher than required by the guideline in one concentration of the test item tested at the end of the test. However, the results of the test have not been impacted.

The $LOEC_{Reproduction}$ value for Carbendazim tested as reference item was 1.0 mg a.s./kg d.wt.s. Significant reductions in reproduction compared to the control was found at the concentrations of 1, 3 and 5 mg a.s./kg d.wt.s. The observed effect is within the expected range from literature. The effects of Carbendazim confirmed the suitable sensitivity of the test system.

Statistical analysis showed no significant difference (Dunnett's test; 2-sided, $p \leq 0.05$) concerning biomass development of adult worms after 28 days between the control and all concentrations of BYI 08330-Enol. Therefore, the $NOEC_{Biomass}$ was considered to be ≥ 1000 mg p.m./kg d.wt.s. (dry weight soil). The $LOEC_{Biomass}$ could not be determined and was regarded as > 1000 mg p.m./kg d.wt.s.
Statistical analysis (Williams test; 1-sided, $p \leq 0.05$) showed a significant difference concerning the number of juveniles between the control and the concentrations of 316 and 1000 mg p.m./kg d.wt.s. Therefore, the $NOEC_{Reproduction}$ was determined as 100 mg p.m./kg d.wt.s. and accordingly the $LOEC_{Reproduction}$ was determined as 316 mg p.m./kg d.wt.s. The EC_{50} value (Reproduction) was calculated by Probit analysis using Linear Max. Likelihood Regression as 1268.9 mg p.m./kg d.wt.s. (95% confidence limits: 1007.2 – 1859.5 mg p.m./kg d.wt.s.).

Effects on mortality, biomass and reproduction of *Eisenia fetida*

Test item	BYI 08330-enol		
Test object	*Eisenia fetida*		
Exposure	artificial soil, 56 days		
Concentration [mg p.m./kg d.wt.s.]	Adult mortality [%]	Biomass [% of initial weight]	Number of juveniles [% of control]
Control	0.0	134.7	100
10	0.0	133.7	89.9
32	0.0	140.0	90.0
100	0.0	134.5	87.7
316	2.5	142.6	76.0*
1000	0.0	141.5	51.4*
NOEC [mg p.m./kg d.wt.s.]	-	≥ 1000	100
LOEC [mg p.m./kg d.wt.s.]	-	> 1000	316
EC_{50} [mg p.m./kg d.wt.s.]	-	-	1268.9

p.m. = pure metabolite
- not applicable
* significantly different to control (Williams test; 1-sided, $p \leq 0.05$)

B. Observations

Most of the food was consumed by the worms in all treatments 7 and 14 days after application of the test item. On days 21 after application half of the food, and on day 28 after application half of the food or less than half of the food was consumed by the worms in all treatments. However, the reduction in food consumption observed was not treatment related. No other effects on behaviour or morphology of the adult earthworms were observed.
0% mortality was observed in the control and 0 - 2.5% mortality at all concentrations of the test item tested. Therefore, an LC_{50} value for mortality could not be calculated but was regarded as > 1000 mg p.m./kg d.wt.s.

CONCLUSION

The $LC_{50\ Mortality}$ was estimated as > 1000 mg p.m./kg d.wt.s.
The $NOEC_{Biomass}$ was ≥ 1000 mg p.m./kg d.wt.s.
The $LOEC_{Biomass}$ was > 1000 mg p.m./kg d.wt.s.
The $NOEC_{Reproduction}$ was determined as 100 mg p.m./kg d.wt.s.
The $LOEC_{Reproduction}$ was determined as 316 mg p.m./kg d.wt.s.
The EC_{50} was determined as 1268.9 mg p.m./kg d.wt.s. (95% confidence limits: 1007.2 – 1859.5 mg p.m./kg d.wt.s.).
The data corresponded to nominal concentrations.

IIA 8.10 Effects on soil microbial activity

IIA 8.10.1 Nitrogen transformation

Report:	**KIIA 8.10.1/01, [redacted]; 2004**
Title:	BYI 08330 tech.: Determination of effects on nitrogen transformation in soil. Date: 2004-09-01
Organisation:	[redacted], Germany
Report No.:	LKC-N-32/04; M-087831-01-1
Publication:	Unpublished
Dates of experimental work:	May 11, 2004 - June 16, 2004
Guidelines:	OECD No. 216; adopted: 21st January 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test.
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The objective of the test was to determine the influence of BYI 08330 tech. on nitrogen transformation in an agricultural soil.

A loamy sand soil was exposed for 28 d to concentrations of 0.13 mg and 1.32 mg BYI 08330 tech./kg dry weight soil. Application rates were equivalent to 0.099 kg and 0.99 kg BYI 08330 tech./ha, respectively. Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.

The test item did not cause a change in the soil pH.

During the 28-day test, it was found that 0.13 mg BYI 08330 tech./kg dry weight soil (equivalent to 0.096 kg a.s./ha) and the 10-fold overdose of the compound had no influence on nitrogen transformation.

MATERIAL AND METHODS

A Materials

1. Test material	**BYI 08330 tech.**
Description	**Not stated in the report**
Lot/batch No.	**Batch no.: Mix-Batch 08045/0014** **TOX no.: 06344-01**
Purity	**97.4%**
Stability of test compound	**Approved until 2004-06-04 when stored at room temperature**
2. Vehicle and/or positive control	**Quartz sand** **Sodium chloride was used as toxic reference in non-GLP tests**
3. Test object	
Soil	**Loamy sand soil with non-target soil microorganisms**

Collection	**The soil was collected on April 13, 2004 at a depth of 0 - 20 cm, passed through a 2 mm sieve and stored at 4 ± 2°C.**
Source	**The soil used is from field plot F on the Bayer AG's experimental farm [redacted], Germany. The [redacted] farm is located at latitude [redacted] and longitude [redacted].**
Soil history	**Plant protection chemicals have not been used on this field since 1981. The plot has been under grass and has not been treated with fertilizers since 1996. On March 07, 2000 the plot was ploughed and then freshly planted with grass.**
Characterisation of the soil	**The particle size distributions were determined annually. The carbon content, the nitrogen content and the cation exchange capacity of the soil was measured annually as well. Soluble nitrogen quantities and the carbon content of the metabolically active microbial biomass in the soil were determined at the start of the test.**
Environmental conditions	
Temperature	**20 ± 2°C**
Photoperiod	**permanent dark**

B Study design and methods

1. In life dates **May 11, 2004 - June 16, 2004**

2. Experimental treatments

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 08330 tech. (0.13 mg or 1.32 mg/kg dry weight soil). The samples were mixed with pulverized lucerne-grass-green meal (5 g/kg dry weight soil) and quartz sand in 3 L polyethylene containers. The Lucerne-grass-green meal, which stimulated the nitrogen transformation was obtained from Hoeveler Kraftfutter, Langenfeld, Germany. The content was 40.6% C_{total}, 0.05% C_{inorg} and 2.5% N (sieve size 2 mm).

After mixing, soil samples equivalent to 300 g dry weight were poured into 500 mL brown glass bottles and these were closed with parafilm. Three replicates were prepared per treatment. The soil was held in the dark at 20 ± 2°C and about 40% of the maximum water holding capacity (WHC_{max}).

3. Observations

Immediately after treatment and after 7, 14 and 28 days a moist sample, corresponding to 10 g dry weight, was removed from each replicate and extracted with 1M KCl. Subsequently soil particles were removed by filtration and the extracts analysed photometrically for their content of ammonium, nitrite and nitrate plus nitrite. The measured values of ammonium, nitrate and nitrite were

divided by their molecular weight and expressed as ammonium-N, nitrate-N and nitrite-N respectively.
The pH-values were measured at the beginning and end of the tests (1M KCl).
The % 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.
Homogeneity of variances was determined by F-test (significance level 5%).
Depending on the results of the F-Tests, the appropriate T-Tests were performed.
The statistical calculations were carried out using Microsoft Excel 97 software.

RESULTS AND DISCUSSION

A. Findings

Soil characteristics

The characteristics for the used soil were listed in the following tables:

Organic carbon content, nitrogen content and cation exchange capacity

Biomass		% C			% N	CEC
(a)	(b)	org. (c)	inorg.	total		(d)
393	6.1	0.649	0.001	0.7	0.10	6.4

(a) Biomass = mg microbial C/kg dry weight soil
(b) Biomass = % of soil organic carbon (c) content
Calculation of (b): (a) X 100/((c) x 10 x 1000) = (a)/((c) x 100)
(d) CEC = Cation Exchange Capacity in meq/100 g dry weight soil

Particle Size Distribution (µm) in Weight %						
2000-630	630-200	200-63	63-20	20-6.3	6.3-2.0	2.0-0
4.0	52.5	21.6	6.9	4.3	4.4	6.3

The pH values at the beginning and the end of the study in untreated and treated samples were between 5.6 and 5.9 – 6.0, so the test item did not cause a change in the soil pH.

Validity criteria and reference substance

The validity criteria according to guideline were met as the highest coefficient of variation (CV) between nitrate-N concentrations in replicate control samples was 11% (14 days after treatment) and thus did not exceed the recommended limit ≤ 15%.
Sodium chloride was used as a reference standard. In tests with the agricultural soil described above, 16 g NaCl/kg dry weight soil had a distinct and long-term (> 28 days) influence on microbial mineralization of nitrogen.

Nitrogen Transformation

Nitrogen transformation in a loamy sand soil treated with BYI 08330 and amended with lucerne-grass-green meal (mean value of 3 soil replicates ± standard deviation)

Days after treatment	Application rate [mg BYI 08330 tech./kg d.wt.s.]					
	0		0.13		1.32	
	mg Nitrogen (N)/kg d.wt.s.		mg Nitrogen (N)/kg d.wt.s.		mg Nitrogen (N)/kg d.wt.s.	
	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N
0	3.27 ± 0.00	28.34 ± 0.41	3.42 ± 0.17	30.07 ± 0.55	3.37 ± 0.21	30.00 ± 0.22
7	1.93 ± 0.08	13.35 ± 1.18	1.95 ± 0.07	14.76 ± 0.65	1.93 ± 0.13	13.32 ± 1.02
14	1.56 ± 0.07	13.46 ± 1.47	1.58 ± 0.06	13.77 ± 1.24	1.76 ± 0.33	13.6 ± 0.54
28	1.48 ± 0.14	26.02 ± 1.46	1.57 ± 0.02	25.94 ± 0.76	1.71 ± 0.18	28.19 ± 1.22

Nitrite formation was not detected in any of the soil samples.

The highest difference between nitrate-N rates per day of control and the one-fold-treated soil samples was 980% (7-14 days after soil treatment). At the end of the test (14-28 days after soil treatment), the difference between nitrate-N rates per day of control was 3%.

The highest difference between nitrate-N rates per day of control and of the 10-fold-overdose-treated soil samples was 143% (7-14 days after soil treatment). At the end of the test (14-28 days after soil treatment), the difference between nitrate-N rates per day of control was 16%.

The high percentage quotation in the time interval 7-14 days after soil treatment of both, one-fold and 10-fold dose emerged from the low nitrate rate/day in this time interval. After an exposure of 28 days the difference in the nitrate rates does not exceed the trigger value of 25% (as given in the guidelines).

Effects on non-target soil micro-organisms

Test item	**BYI 08330 (tech.)**	
Test object	**Soil Micro-organisms**	
	Nitrogen transformation (loamy sand soil)	
Exposure	**28 d**	
mg test item/kg dry weight soil	**0.13**	**1.32**
kg test item (kg a.s.)/ha (equivalent)	**0.099 (0.096) (one-fold rate)**	**0.99 (0.96) (10-fold rate)**
Final result after 28 days	**Difference to control < 25%**	**Difference to control < 25%**

CONCLUSION

During the 28-day test, the one-fold field rate of spirotetramat 0.096 kg a.s./ha (equivalent to 0.13 mg a.s./kg dry weight soil) and the 10-fold overdose of the compound had no influence on nitrogen transformation in a loamy sand soil amended with lucerne-grass-green meal.

IIA 8.10.2 Carbon mineralization

Report:	**KIIA 8.10.2/01, [redacted]; 2004**
Title:	BYI 08330 tech.: Determination of effects on carbon transformation in soil. Date: 2004-09-01
Organisation:	[redacted], Germany
Report No.:	LKC-C-30/04; M-087840-01-1
Publication:	Unpublished
Dates of experimental work:	May 11, 2004 - June 08, 2004
Guidelines:	OECD No. 217, Adopted: 21st January 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Carbon Transformation Test.
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The objective of the test was to determine the influence of BYI 08330 tech. on carbon transformation (glucose stimulated respiration) in an agricultural soil.

A loamy sand soil was exposed for 28 d to concentrations of 0.13 mg and 1.32 mg BYI 08330 tech./kg dry weight soil (application rates were equivalent to 0.099 kg and 0.99 kg BYI 08330 tech./ha, respectively). Glucose was added to the soil samples (2 g/kg dry weight soil) to induce maximum respiration rate.

The test item did not cause a change in the soil pH.

During the 28-day tests, the recommended field rate of spirotetramat (0.096 kg a.s./ha) and the 10-fold overdose of the test item had no influence on carbon transformation after addition of glucose.

MATERIAL AND METHODS

A Materials

1. Test material — **BYI 08330 tech.**

Description	**Not stated**
Lot/batch No.	**Batch no.: Mix-Batch 08045/0014** **TOX no.: 06344-01**
Purity	**97.4%**
Stability of test compound	**Approved until 2004-06-04 when stored at room temperature**

2. Vehicle and/or positive control — **Quartz sand**

Sodium chloride was used as toxic reference in non-GLP tests

3. Test object

Soil	**Loamy sand soil with non-target soil microorganisms**

Collection	**The soil was collected on April 13, 2004 at a depth of 0 - 20 cm, passed through a 2 mm sieve and stored at 4 ± 2°C.**
Source	**The soil used is from field plot F on the Bayer AG's experimental farm [redacted], Germany. The [redacted] farm is located at latitude [redacted] and longitude [redacted]**
Soil history	**Plant protection chemicals have not been used on this field since 1981. The plot has been under grass and has not been treated with fertilizers since 1996. On March 07, 2000 the plot was ploughed and then freshly planted with grass.**
Characterisation of the soil	**The particle size distributions were determined annually. The carbon content, the nitrogen content and the cation exchange capacity of the soil was measured annually as well. Soluble nitrogen quantities and the carbon content of the metabolically active microbial biomass in the soil were determined at the start of the test.**
Environmental conditions	
Temperature	**20 ± 2 °C**
Photoperiod	**permanent dark**

B Study design and methods

1. In life dates **May 11, 2004 - June 08, 2004**

2. Experimental treatments

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 08330 tech. (0.13 mg or 1.32 mg/kg dry weight soil). The samples were intensely mixed in 3 L polyethylene containers.

After mixing, soil samples equivalent to 350 g dry weight were poured into 500 mL brown glass bottles and these were closed with parafilm. Three replicates were prepared per treatment. The soil was held about 40% of the maximum water holding capacity (WHC_{max}).

Preliminary tests were used to determine the amounts of glucose needed to induce a maximum respiration rate in control samples of the soil. The soil required 2000 mg glucose/kg dry weight.

3. Observations

The pH-values were measured at the beginning and end of the tests.

To determine the influence of BYI 08330 tech. on glucose stimulated soil respiration, moist samples (equivalent to 25 g dry weight) were taken from each treatment on day 0, and after 7, 14 and 28 days of incubation. The samples were mixed with glucose (2000 mg/kg dry weight) to induce maximum respiration rates

and poured into plastic cylinders (3 cm diameter, 23 cm long). The cylinders were connected to a gas analyzer and the quantities of carbon dioxide released per hour per kg dry weight soil were measured for at least 12 hours.
The % differences in the quantities of CO_2/h/kg d.wt.s. 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 F-test (significance level 5%).
Depending on the results of the F-Tests, the appropriate T-tests were performed. In the T-tests the sum of 12 hours of the mean values of CO_2/h/kg dry weight from control soils and treated soils were compared. The statistical calculations were carried out using Microsoft Excel 97 software.

RESULTS AND DISCUSSION

A. Findings

Soil characteristics

The characteristics for the used soil were listed in the following tables:

Organic carbon content, nitrogen content and cation exchange capacity

Biomass (a)	(b)	% C org (c)	inorg	total	% N	CEC (d)
393	6.1	0.649	0.001	0.7	0.10	6.4

(a) Biomass = mg microbial C/kg dry weight soil
(b) Biomass = % of soil organic carbon (c) content
Calculation of (b): (a) X 100/((c) x 10 x 1000) = (a)/((c)x100)
(d) CEC = Cation Exchange Capacity in meq/100 g dry weight soil

Particle Size Distribution (µm) in Weight %						
2000-630	630-200	200-63	63-20	20-6.3	6.3-2.0	2.0-0
4.0	52.5	21.6	6.9	4.3	4.4	6.3

The pH values at the beginning and the end of the study in untreated and treated samples were between 5.5 - 5.6, so the test item did not cause a change in the soil pH.

Validity criteria and reference substance

The validity criteria according to guideline were met as the highest coefficient of variation (CV) between carbon dioxide concentrations in replicate control samples was 2.3% (7 days after treatment) and thus did not exceed the recommended limit ≤ 15%.
Sodium chloride was used as a reference standard. In tests with the agricultural soil described above, 16 g NaCl/kg dry weight soil had distinct and long-term (> 28 days) influence on microbial mineralization of carbon.

Carbon Transformation

Carbon transformation in a loamy sand soil treated with BYI 08330 and amended with glucose expressed as the sum of 12 hours of the mean values of CO_2/h/kg dry weight (mean value of 3 soil replicates ± standard deviation)

Days after treatment	Application rate [mg BYI 08330 tech./kg d.wt.s.] 0		0.13		1.32	
	mg CO_2/ 12 h/kg d.wt s.	% of control	mg CO_2/ 12 h/kg d.wt s.	% of control	mg CO_2/ 12 h/kg d.wt s.	% of control
0	268.3 ± 4.2	100	251.7 ± 7.7	94	248.7* ± 0.6	93
7	284.6 ± 6.5	100	256.9* ± 3.1	90	253.8* ± 5.1	89
14	290.5 ± 6.4	100	267.8* ± 5.9	92	269.5* ± 4.6	93
28	264.5 ± 1.7	100	247.3* ± 5.2	94	245.9* ± 2.6	93

* Significant difference between treated and untreated soil samples (T-test with 5% probability of error).

The highest difference between carbon dioxide concentration in control and one-fold treated soil samples was 10% (7 days after soil treatment). The highest difference between carbon dioxide concentration in control and treated soil samples with the 10-fold overdose of the compound was 11% (7 days after soil treatment).
After an exposure of 28 days the difference in the carbon dioxide production does not exceed the trigger value of 25% (as given in the guidelines).

Effects on non-target soil microorganisms

Test item	BYI 08330 (tech.)
Test object	Soil Microorganisms
	Carbon-Transformation (loamy sand soil)
Exposure	28 d
mg test item/kg dry weight soil	0.13	1.32
kg test item (kg a.s.)/ha (equivalent)	0.099 (0.096) (one-fold rate)	0.99 (0.96) (10-fold rate)
Final result after 28 days	Difference to Control < 25%	Difference to Control < 25%

CONCLUSION

During the 28-day tests, the one-fold field rate of spirotetramat 0.096 kg a.s./ha (equivalent to 0.13 mg a.s./kg dry weight soil) and the 10-fold overdose of the compound had no influence on respiration after adding glucose to the loamy sand soil.

IIA 8.10.3 Rates of recovery following treatment

Studies on recovery following treatment are not required as spirotetramat is not intended for use in products for soil sterilisation and has no long-term effects on soil micro-organisms.

IIA 8.11 Effects on marine and estuarine organisms*

Four studies with spirotetramat were performed on marine or estuarine organisms and are summarised under IIA 8.11.1. However, within EU dossiers, no risk assessments are performed for saltwater organisms.

IIA 8.11.1 Marine or estuarine organisms acute toxicity LC50/EC50*

Report:	**KIIA 8.11.1/01, [redacted] & [redacted], 2005**
Title:	Acute Toxicity of BYI 08330 Technical to the Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions Date: 2005-07-27
Organisation:	Bayer CropScience, Ecotoxicology, South Metcalf, Stilwell, Kansas
Report No.:	EBFNX006; M-285363-01-1
Publication:	Unpublished
Dates of experimental work:	April 25, 2005 - April 29, 2005 (biological work)
Guidelines:	OECD Guideline 203 OPPTS Guideline 850.1075 FIFRA 72-3
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

A 96-hour flow-through test was conducted to determine the acute toxicity of BYI 08330 technical to the Sheepshead minnow (*Cyprinodon variegatus*). The primary endpoint for acute toxicity was mortality, expressed as a 96-hour median lethal concentration (LC_{50}). Sublethal and behavioural effects were also assessed during the course of the study, expressed as the No Observed Effect Concentration (NOEC) and the Lowest Observed Effect Concentration (LOEC).

Juvenile *Cyprinodon variegatus* were exposed under flow-through conditions for approximately 96 hours to the following nominal concentrations (Day 0, 2 and 4 mean measured): control (<0.03), solvent control (<0.03), 0.63 (0.52), 1.25 (1.17), 2.50 (2.52), 5.0 (4.54) and 10 (9.77) mg a.s. (mean measured a.s.)/L.

The physical/chemical properties of the testing water, dissolved oxygen, temperature, pH values and salinity were measured. Due to analytical measurements all results are given as mean measured test concentrations of the active substance.

Based on nominal concentrations the LC_{50} was calculated by at 1.96 mg a.s./L. The NOEC and LOEC were empirically determined based on mortalities and sublethal effects at 0.52 mg a.s./L and 1.17 mg a.s./L, respectively.

* No EC data requirement (the OECD point concerned is not covered by or part of an EC point according to Council Directive 91/414/EEC. Hence, data/documents do not need to be submitted.

* No EC data requirement (the OECD point concerned is not covered by or part of an EC point according to Council Directive 91/414/EEC. Hence, data/documents do not need to be submitted.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**White powder**
Lot/batch No.	**Batch no.: 08045/0014 (mix-batch)**
Purity	**97.99%**
Stability of test compound	**Expiration date: April 18, 2007 when stored at ambient temperature**
2. Vehicle and/or positive control	**Acetone was used as solvent for the test item (0.10 mL/L).**
3. Test animals	
Species	***Cyprinodon variegatus* (Sheepshead minnow), mean body length 17.2 ± 1.4 mm, mean body weight 0.13 ± 0.03 g, biomass loading was 0.03 g fish/L test medium**
Age	**Juvenile**
Source	[redacted]
	Date of arrival: April 8, 2005
Acclimation period	**> 14 days under test conditions. Fish were feed daily (with Tetramin and brine shrimp) except for the last 48 hours prior to testing. No mortalities occurred during 48 hours prior to testing, no treatments for disease was necessary.**
Environmental conditions	
Temperature	**22 ± 1°C**
Photoperiod	**16:8 hours, 30 minutes light/dark transition period, 689 to 1023 lux**

B Study design and methods

1. In life dates **April 25, 2005 - April 29, 2005**

2. Experimental treatments

Test vessels consisted of glass with a volume of 22 L (34.3 x 21.6 x 30.5 cm). Testing volume used was 18 L (34.3 x 21.6 x 24.3 cm). Test tanks were randomly placed in test bath. Test vessel loading was 0.03 g fish/L test medium passing through the tank each day.

Per concentration level, 1 replicate containing 20 juvenile organisms was established. Fish were impartially distributed (two at a time) to each test chamber until twenty fish were distributed to each test chamber. Fish were not fed during the test and test solutions were not aerated during the test.

The test system was a flow-through system, consisting of a modified Mount-Brungs proportional diluter system with approx. 5 renewals per day. Testing and culture water was reverse osmosis produced water or processed water (blended spring and

reverse osmosis). The appropriate volume of dilution water and toxicant solution was blended and delivered by two syringe pumps to the test aquaria to create the 0.63, 1.25, 2.5, 5.0 and 10.0 mg a.s./L test solutions. The diluter system was calibrated to an accuracy of ± 10% prior to test initiation.

3. Observations

Survival (mortality) and sublethal behavioural effects were examined after approx. 4, 24, 48, 72 and 96 hours by enumeration and observation, respectively.
For analytical verification samples were analysed for the actual concentration of BYI 08330 present in the test medium at all treatment levels and the controls after 0, 48 and 96 hours. Temperature was measured hourly, pH values were measured at days 0 and 4, dissolved oxygen and salinity were measured at days 0, 2 and 4.
The 96-hour LC_{50} value and was calculated by a LC_{50} computer program developed by [redacted] et al. (1984) using the moving average statistical method. The method selected was determined by the characteristics of the data, i.e., the number of concentrations in which mortalities between 0 and 100 percent occurred and the 95% confidence intervals ([redacted], 1977). The NOEC and LOEC were empirically determined based upon observation data including lethal and sublethal effects.

RESULTS AND DISCUSSION

A. Findings

Mean measured recoveries were calculated based on results of samples collected on day 0, 2 and 4. All subsequent observations will refer to mean measured concentrations of the test solutions.

Based on analytical determination of BYI 08330 mean measured values between 82 to 101% of nominal test concentrations were found in all exposure levels over the whole testing period of 96 h.

Dissolved oxygen concentrations ranged from 67 to 87% oxygen saturation (5.3 – 6.9 mg/L), the pH values ranged from 7.7 to 8.4, the water temperature ranged from 21.0 to 22.5°C and salinity ranged from 17 to 18‰ in all aquaria over the whole testing period.

Acute toxicity to fish

Test substance	BYI 08330 techn. a.s.
Test object	Sheepshead Minnow
Exposure	96-hour, flow-through
LC_{50}	1.96 mg a.s./L
Lowest Concentration With an Effect (LOEC)	1.17 mg a.s./L
Highest Concentration Without Toxic Effect (NOEC)	0.52 mg a.s./L
Toxic Threshold Effect Concentration, TEC (Geometric mean of NOEC and LOEC)	1.30 mg a.s./L

B. Observations

Exposure time	4 h		24 h		48 h		72 h		96 h	
Test level mg a.s./L (mean measured)	Dead	Obs	Dead	Obs	Dead	Obs	Dead	Obs	Dead	Obs
control	0	20 N	0	20 N	0	20 N	0	20 N	0	20 N
solvent control	0	20 N	0	20 N	0	20 N	0	20 N	0	20 N
0.52	0	20 N	0	20 N	0	20 N	0	20 N	0	20 N
1.17	0	20 N	0	20 N	0	20 N	0	18 N, 1LE, 3LE,OB	2	17 N, 1LE
2.52	0	20 N	0	20 N	0	18 N, 1 LE, 3LE,OB	7	7 N, 3 LE, 3LE,OB	12	2 N, 3 LE, 3LE,OB
4.54	0	20 N	2	15 N, 3LE,OB	20	-	20	-	20	-
9.77	0	20 N	20	-	20	--	20	-	20	-

Dead = Cumulative number of dead Obs. = Observations (no. of individuals alive observation)
N = Normal Sol. = Solvent
LE = Loss of Equilibrium OB = On Bottom

CONCLUSION

The LC_{50} was calculated by moving average statistical method at 1.96 mg a.s./L (based on nominal concentrations). The NOEC and LOEC were empirically determined based on mortalities and sublethal effects at 0.52 mg a.s./L and 1.17 mg a.s./L, respectively.

Report: **KIIA 8.11.1/02, [redacted], M.A.; 2005**
Title: BYI 08330 - Acute Toxicity to Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Conditions
Date: 2005-06-15
Organisation: Springborn Smithers Laboratories, Wareham, Massachusetts
Report No.: EBFNX011; M-237677-01-1
Publication: Unpublished
Dates of experimental work: March 24, 2005 - March 28, 2005 (biological work)
Guidelines: FIFRA Guideline 72-3
OPPTS Guideline 850.1025 (Draft)
Deviations: no major deviations
GLP: yes (certified laboratory)

Executive summary

The aim of this study was to estimate the acute toxicity of BYI 08330 to Eastern oysters (*Crassostrea virginica*) under flow-through conditions. Reduction of shell deposition was used as the indicator of toxicity. 20 oysters were placed in each test aquarium (40 per treatment level

and the control) and exposed to five test concentrations of nominal 0.19, 0.38, 0.75, 1.5 and 3.0 mg a.s./L, a dilution water control and a solvent control.
As measurements show, the physical/chemical properties of the test water (pH, temperature, oxygen-saturation, salinity) were unaffected by the concentrations of the test substance and remained within acceptable levels for the survival and growth of Eastern oysters. Due to analytical measurements all results are given as mean measured test concentrations of the active substance.
The 96-hour EC_{50} value was calculated by linear regression to be 0.85 mg a.s./L. The No-Observed-Effect Concentration (NOEC) was determined to be 0.33 mg a.s./L, based on Williams' Test.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**Not specified in the report**
Lot/batch No.	**Batch no.: 08045/0014 (mix-batch)**
Purity	**97.1%**
Stability of test compound	**2005-05-05**
2. Vehicle and/or positive control	**Dimethylformamide (DMF) was used as solvent; Natural seawater was used as dilution and control water.**
3. Test animals	
Species	**Eastern oyster (*Crassostrea virginica*) of similar age and a mean valve height of 37 ± 5 mm**
Age	**Not specified**
Source	**Circle C Oysters, Ridge, Maryland, US**
Acclimation period	**Oysters were held in a polypropylene tray through which seawater flowed continuously for 14 days prior to testing. The seawater had a temperature range of 14 to 19ºC, a pH range of 7.5 to 7.8 and a dissolved oxygen concentration range of 97 to 105% of saturation. At their source, the oysters were collected from water which had a salinity of approximately 9‰. During the acclimation period, the salinity was gradually increased from 16 to 32‰.**
Environmental conditions	
Temperature	**21 ± 2°C**
Photoperiod	**16 : 8 hours light : darkness**

B Study design and methods

1. In life dates	**March 24, 2005 - March 28, 2005**
2. Experimental treatments	

Prior to testing, 3 to 5 mm of the new peripheral shell growth of each oyster was removed.
During the culture period, oysters were fed a supplementary algal diet of *Tetraselmus maculata* prepared in seawater from a concentrate. No mortality was observed among the oyster population during the seven days before test initiation.
During the exposure, the oysters received supplemental feedings of algae (*Tetraselmus maculata*).
The exposure of oysters was initiated by impartially selecting and placing 20 oysters in each test aquarium (40 per treatment level and the control). Oysters were spaced equidistant from one another with their valve inflow openings facing toward the flow of water from the circulator tube.
The test was conducted using an exposure system consisting of a constant flow diluter with a dilution factor of 50% between concentrations, a temperature-controlled water bath and a set of 14 exposure aquaria. The function of the diluter system (e.g., flow rates, stock solution consumption) was monitored daily and a visual check of the systems' function was performed twice daily. The test system was designed to provide five test concentrations of BYI 08330, a dilution water control and a solvent control. All treatment levels and the controls were maintained in duplicate. Based on preliminary testing, the nominal concentrations selected for the definitive test were 0.19, 0.38, 0.75, 1.5 and 3.0 mg a.s./L.
Test vessels consisted of glass aquaria measuring 49.5 x 25.5 x 29 cm; a test solution volume of approximately 18 L was maintained. The flow of exposure solution to each aquarium (75 mL/minute) provided approximately 6.0 solution volume replacements every 24 hours in order to provide a 90% solution replacement rate of approximately 9 hours. In addition, the contents of each aquarium were continuously circulated aiding in evenly distributing the algae fed to the oysters and in mixing the flow of test solution throughout each aquarium.
Natural seawater was used as dilution and control water during this study.

3. Observations

Biological observations (e.g., visible abnormalities, such as excessive mucous production or a failure to siphon and feed, as evidenced by a lack of fecal and pseudofecal production) and observations of the physical characteristics (the pH, temperature, salinity, and dissolved oxygen concentration) of the test solutions were made at test initiation and at each subsequent 24-hour interval until termination of the test. In addition, temperature was monitored continuously in one replicate of the 0.19 mg a.s./L (nominal) test solution.
Sublethal effects were determined by a comparison of the performance and appearance of the exposed oysters to that of the control oysters. After 96 hours of exposure, the oysters were removed from the test aquaria and the new shell growth was measured microscopically to the nearest 0.1 mm using a calibrated micrometer.
Routine analyses were conducted periodically on representative samples of the water source for the presence of pesticides, PCBs, and toxic metals.

For analytical verification, samples were removed from one replicate solution of each treatment level and the controls at each sampling interval (= test initiation and termination).
The 96-hour EC_{50} value and 95% confidence intervals are determined by fitting the untransformed or transformed (i.e., growth data as percent reduction transformed to probit, concentrations transformed to log concentration) data to a best fit linear regression curve based on least squares. A computer program developed at Springborn Smithers is used to assist in these computations. The No-Observed-Effect Concentration (NOEC) for the 96-hour exposure period was statistically determined by using Williams' Test.

RESULTS AND DISCUSSION

A. Findings

The protocol states that the total dissolved oxygen concentration will not be allowed to drop below 60% of saturation (4.5 mg/L at 20ºC and 32‰ salinity) during the test. During the final 24 hours of the exposure, dissolved oxygen levels ranged from 52 to 96% of saturation.
The protocol also states that the test solution temperature will be maintained at 20 ± 2ºC throughout the exposure. During the final 24 hours of this test, the maximum temperature was recorded on the minimum/maximum thermometer to be 23ºC.
However, these deviations did not have a negative impact on the results or interpretation of the study.

Dilution water was natural seawater, with salinity of 31 to 32‰ and pH of 8.1. Based on the analysis for pesticides, the water source was considered to be of acceptable quality since all analyte concentrations were below levels of concern.

Measured concentrations at both the 0- and 96-hour intervals followed the appropriate dose gradient and recoveries decreased slightly between test initiation and test termination. The mean measured concentrations ranged from 59 to 100% of nominal concentrations and defined the treatment levels tested as 0.20, 0.33, 0.55, 0.89 and 2.1 mg a.s./L.

The growth of the control and solvent control groups during this study was within the historical range (0.5 to 4.5 mm) compiled at Springborn Smithers. At test termination, no mortality or adverse effects were observed among oysters at any of the treatment levels tested. Following 96 hours of exposure, 3, 31, 54 and 89% reduction in shell growth was observed among oysters exposed to the 0.33, 0.55, 0.89 and 2.1 mg a.s./L treatment levels, respectively. The remaining treatment level tested (0.20 mg a.s./L) exhibited a positive response compared the pooled control. A concentration-response curve is presented in the report.

CONCLUSION

The 96-hour EC_{50} value was calculated by linear regression to be 0.85 mg a.s./L, with 95% confidence intervals of 0.59 to 1.3 mg a.s./L. The No-Observed-Effect Concentration (NOEC) was determined to be 0.33 mg a.s./L, based on Williams' Test.

Report:	**KHA 8.11.1/03, [redacted].; 2005**
Title:	Acute Toxicity to Mysids (*Americamysis bahia*) Under Flow-Through Conditions.
	Date: 2005-01-6
Organisation:	Springborn Smithers Laboratories, Wareham, Massachusetts
Report No.:	EBFNX010; M-270200-01-1
Publication:	Unpublished
Dates of experimental work:	September 24, 2004 - September 28, 2004 (biological work)
Guidelines:	FIFRA Guideline 72-3 OPPTS Draft Guideline 850.1035
Deviations:	None

GLP: yes (certified laboratory)

Executive summary
The aim of this study was to estimate the acute toxicity of BYI 08330 to Mysids (*Americamysis bahia*) under flow-through conditions for 96 hours. Survival was used as the indicator of toxicity.
20 juvenile mysids were placed in test vessels (10 mysids per replicate) and exposed to five test concentrations of nominal 0.63, 1.3, 2.5, 5.0 and 10 mg a.s./L (corresponding to mean measured concentrations of 0.73, 1.2, 2.6, 4.6 and 9.0 mg a.s./L), a dilution water control and a solvent control. As measurements show, pH, temperature and salinity remained within acceptable levels for the survival and growth of mysids. Dissolved oxygen levels decreased over the exposure period but were maintained above 60% saturation throughout the exposure. The analytical content of a.s. was determined and all results are given as mean measured test concentrations of the active substance.
The 96-hour LC_{50} (corresponding 95% confidence intervals) was estimated by binomial probability to be 5.5 mg a.s./L (1.2 to 9.0 mg a.s./L). The 96-hour No-Observed-Effect Concentration (NOEC) was determined to be 1.2 mg a.s./L.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) tech.**
Description	**Expiration date: November 4, 2004**
Lot/batch No.	**Batch no.: 08045/0014 (mix-batch)**
Purity	**97.2%**
Stability of test compound	**Not specified in the report**
2. Vehicle and/or positive control	**Solvent: dimethylformamide, DMF**
	Filtered seawater (salinity adjusted with laboratory well water; salinity: 21 to 22‰, pH 7.9 to 8.1) was used as dilution water.
3. Test animals	
Species	**Mysids (*Americamysis bahia*)**
Age	**5- to 6-days old**
Source	**Obtained from laboratory cultures maintained at Springborn Smithers. Brood stock was originally obtained from [redacted].**
Acclimation period	**During the 14-day period prior to test initiation mysids were cultured in a 76 L glass aquaria with a filtration system providing artificial seawater (salinity: 20 to 24‰, pH 8.2 to 8.3).**
Environmental conditions	
Temperature	**25 ± 1 °C**

Photoperiod	**16:8 hours, light intensity: 39 to 52 footcandles (420 to 560 lux)**

B Study design and methods

1. In life dates September 24, 2004 - September 28, 2004

2. Experimental treatments

Mysids were cultured in a 76 L glass aquaria with a closed-loop recirculating filtration system providing artificial seawater to the aquaria. The artificial seawater in the aquaria was characterised as having a salinity of 20 to 24‰, and a pH of 8.2 to 8.3 during the 14-day period prior to test initiation. Dissolved oxygen percent saturation ranged from 94 to 97%, and specific conductance ranged from 30,000 to 34,000 µmhos/cm. The culture solutions were maintained at a temperature range of 26 to 27°C. Mysids were fed brine shrimp (*Artemia salina*) nauplii *ad libitum*, twice daily.

Prior to test initiation, mysids (5- to 6-days old) were impartially selected and placed in the test vessels (10 mysids in each of the 2 replicates, 20 mysids per treatment level and the controls).

Test vessels consisted of 1.6 L square glass battery jars with two 2 cm holes drilled approximately 15 cm from the bottom of each jar and covered with 40-mesh screen for drainage. Total solution volume was maintained at 1.4 L. The diluter was calibrated to deliver approximately 50 mL/cycle of newly prepared exposure solution to each replicate test vessel. The flow of exposure solution provided approximately 6.0 solution volume replacements per day in order to provide a 90% test solution replacement rate of approximately 9.0 hours. 2 replicates per level were established. The nominal concentrations selected for the definitive test were 0.63, 1.3, 2.5, 5.0 and 10 mg a.s./L.

The dilution water used during this study was natural seawater and was prepared daily by adjusting the salinity to 20 ± 3‰ with laboratory well water.

3. Observations

Biological observations (e.g., abnormal behaviour or appearance of the test organisms) and observations of the physical characteristics of the test solutions (e.g., precipitate, film on the surface of the test solution, the pH, temperature, salinity, and dissolved oxygen concentration) were made and recorded at test initiation and at each subsequent 24 hour interval. Mortality was defined as lack of movement after gentle prodding with a glass pipette. In addition, temperature was monitored continuously in one replicate of the 10 mg a.s./L solution.

Routine analyses were conducted periodically on representative samples of the food and dilution water source for the presence of pesticides, PCBs, and toxic metals. For analytical verification, samples were removed from one replicate solution of each treatment level and the controls at each sampling interval (= test initiation and termination).

A computer program ([redacted], 1982) was used to calculate the LC_{50} values and 95% confidence intervals. Three statistical methods were available in the computer program: moving average angle analysis, probit analysis and binomial probability.

A. Findings

The results of the water quality parameters (pH, temperature, and salinity) were unaffected by the concentrations of the test substance and remained within acceptable levels for the survival and growth of mysids. Dissolved oxygen levels decreased over the exposure period but were maintained above 60% saturation throughout the exposure. Daily monitoring of the test solutions and continuous temperature monitoring of one replicate of the 10 mg a.s./L established that the exposure solution temperature ranged from 25 to 26°C during the exposure period.

Based on the analysis for pesticides, the food source was considered to be of acceptable quality since all analyte concentrations were below levels of concern. The dilution water used had a salinity of 21 to 22‰ and a pH of 7.9 to 8.1.

Based on mean measured concentrations, the exposure levels ranged from 90 to 120% of nominal concentrations and were defined as 0.73, 1.2, 2.6, 4.6 and 9.0 mg a.s./L. All results reported based on mean measured concentrations.

Acute toxicity to Mysid Shrimp

	LC_{50} [mg a.s./L]	95% Confidence Intervals lower [mg a.s./L]	upper [mg a.s./L]
24-Hour[a]	9.1	6.2	21
48-Hour[b]	6.1	4.6	9.0
72-Hour[c]	4.5	3.7	5.6
96-Hour[b]	5.5	1.2	9.0
NOEC through 96 hours = 1.2 mg a.s./L			

[a] LC_{50} value and corresponding 95% confidence intervals were calculated by probit analysis.

[b] LC_{50} value and corresponding 95% confidence intervals were estimated by binomial probability.

[c] LC_{50} value and corresponding 95% confidence intervals were calculated by moving average angle analysis.

B. Observations

Following 96 hours of exposure, 5, 5, 30, 25 and 100% mortality was observed among mysids exposed to the 0.73, 1.2, 2.6, 4.6 and 9.0 mg a.s./L treatment levels, respectively. Although mortality of 5% was observed in the two lowest treatment levels tested (0.73 and 1.2 mg a.s./L), this is considered to be within the expected range of naturally occurring variability for acute tests and not toxicant-related. No mortality or sublethal effects were observed among mysids exposed to the controls. Several surviving organisms exposed to the 2.6 mg a.s./L treatment level exhibited erratic swimming behaviour. Several surviving organisms exposed to the 4.6 mg a.s./L treatment level were observed to be lethargic, while two surviving organisms were lethargic and on the bottom of the test vessel.

CONCLUSION

The 96-hour LC_{50} (corresponding 95% confidence intervals) was estimated by binomial probability to be 5.5 mg a.s./L (1.2 to 9.0 mg a.s./L). The 96-hour No-Observed-Effect Concentration (NOEC) was determined to be 1.2 mg a.s./L.

Report:	**KIIA 8.11.1/04, [redacted] & [redacted] 2006**
Title:	Toxicity of BYI 08330 Technical to the Saltwater Diatom *Skeletonema costatum*. Date: 2006-02-23
Organisation:	Bayer CropScience, Ecotoxicology, South Metcalf, Stilwell, Kansas
Report No.:	EBFNX009; M-271037-02-1
Publication:	Unpublished
Dates of experimental work:	November 23, 2004 – December 16, 2004
Guidelines:	FIFRA Guideline 123-2 (1982) OPPTS Guideline 850.5400 (1996 draft) OECD Guideline 201 (1984, 2004 draft)
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 *Skeletonema costatum* expressed as NOEC, LOEC, and ECx for growth rate of algal biomass (cells per volume). *Skeletonema costatum* were exposed in a multigeneration test for 4 days under static (shaken culture) exposure conditions to the nominal concentrations of 0.256, 0.640, 1.60, 4.00 and 10.0 mg a.s./L in comparison to controls.

The physico/chemical parameters corresponded to the aspired values. Initial measured recoveries were within the range of 94 to 106% of the nominal concentrations. Since the material was not stable in the test system, the toxicity values were calculated based on the initial measured concentrations.

The 72 hour EC_{50} value for growth rate (E_rC_{50}) is 1.55 mg a.s./L with LOEC and NOEC values of 0.62 and 0.24 mg a.s./L, respectively. The 96 hour EC_{50} value for growth rate (E_rC_{50}) is 1.56 mg a.s./L with LOEC and NOEC values of 0.62 and 0.24 mg a.s./L, respectively. The most sensitive 96 hour EC_{50} value for biomass was based on cell density (standing crop). This was calculated to be 0.55 mg a.s./L with LOEC and NOEC values of 0.62 and 0.24 mg a.s./L, respectively.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat BYI 08330 technical**
Description	**Solid white powder**
Lot/batch No.	**Batch no. 08045/0014**
Purity	**97.99%**
Stability of test compound	**Expiration date: April 18, 2007 when stored at room temperature**
2. Vehicle and/or positive control	**Acetone was used as solvent for the test item (0.5 mL/L)**
3. Test animals	
Species	***Skeletonema costatum*, strain CCAP1077/5 (supplier strain synonym)**
Age	**Exponentially growing inoculum**

Source	**Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP), West Booth Bay Harbor, Maine, US**
Acclimation period	**The inoculum was obtained from an in-house 5 day old batch culture held under test conditions**
Environmental conditions	
Temperature	**20 ± 2.0°C**
Photoperiod	**16:8 light : dark; 4300 lux (± 10%)**

B Study design and methods

1. In life dates **January 09, 2006 - January 13, 2006**

2. Experimental treatments

Detailed algae culture records are maintained in the Bayer CropScience Aquatic Toxicology Lab logbook, Kansas.

To ensure that the algae used as inoculum were exponentially growing, an inoculum pre-culture was prepared 5 days before the start of the test and cultivated under the same conditions as in the main test.

The test vessels consisted of 250 mL steril Erlenmeyer flasks, filled with 100 mL nutrient medium (ES media) and an initial cell density of 10,000 cells/mL. They were placed on a tablet rotating 100 rpm to prevent sedimentation of the cells without additional aeration. The source of water was spring water blended with reverse osmosis water to produce soft (40 to 60 mg/L) water. The stock solution contained 5.1028 g BYI 08330 with 250 mL acetone.

The actual study included 3 replicate vessels per test level. The range of test concentrations was set based on the practical limit of solubility for BYI 08330 technical in saltwater. No range finding test was conducted. Nominal concentrations were control, solvent control, 0.256, 0.640, 1.60, 4.00 and 10.0 mg a.s./L. The test duration accounted to 4 days.

3. Observations

The temperature was determined by a continuous measurement in one additional incubated 250 mL flask of water centrally located in the environmental chamber. The measurement interval for salinity, pH and light intensity were day 0 and day 4. Morphological examinations of cells by 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 via light microscope and hemocytometer slide. The limit of detection for the hemocytometer was 1 x 10^4 alga cells/mL. After 72 and 96 hour the growth rate (NOEC and EC_{50}) was determined, additionally after 96 hour the standing crop and cumulative biomass.

For analytical verification samples were analysed for the actual concentration of BYI 08330 present in the test medium at all treatment levels and the controls on day 0 (from batch preparation) and day 4 (composited samples from each level).

The limit of quantitation (LOQ) for the method used (in water by HPLC-UV) was 0.02 µg/L. The method was validated within the current study.

RESULTS AND DISCUSSION

A. Findings

No physical abnormalities were observed in the controls or treatment groups during the study.
The incubation temperature was 19.4 to 21.0°C over the whole period of testing at a light intensity from 3897 to 4682 lux, the pH value ranged from 8.0 to 8.4, the salinity was 27‰.
The analytical findings of BYI 08330 in the treatment levels found on day 0 were 94 to 106% of nominal test concentrations. On day 4 analytical findings of 25 to 46% of nominal test concentrations were found. Therefore all results are based on the initial measured test concentrations.

Acute toxicity to saltwater diatom *Skeletonema costatum*

Test substance	Spirotetramat tech.
Test object	*Skeletonema costatum*
Exposure	96-hour, static
0-72 h E_rC_{50} - growth rate	1.55 mg a.s./L
96 h E_rC_{50} – growth rate	1.56 mg a.s./L
96 h E_bC_{50} – biomass (cell density, standing crop)	0.55 mg a.s./L

CONCLUSION

The 72 and 96-hour growth rates were calculated based on initial measured concentrations. The 72 hour EC_{50} value for growth rate (E_rC_{50}) is 1.55 mg a.s./L with LOEC and NOEC values of 0.62 and 0.24 mg a.s./L, respectively. The 96 hour EC_{50} value for growth rate (E_rC_{50}) is 1.56 mg a.s./L with LOEC and NOEC values of 0.62 and 0.24 mg a.s./L, respectively. The most sensitive 96 hour EC_{50} value for biomass was based on cell density (standing crop). This was calculated to be 0.55 mg a.s./L with LOEC and NOEC values of 0.62 and 0.24 mg a.s./L, respectively.

IIA 8.11.2 Marine/Estuarine fish - salinity challenge*

No data requirement for submission in EU, U.S. or Canada. Hence, data/documents do not need to be submitted.

*** No EC data requirement (the OECD point concerned is not covered by or part of an EC point according to Council Directive 91/414/EEC. Hence, data/documents do not need to be submitted.**

IIA 8.12 Effects on terrestrial vascular plants

Report:	**KIIA 8.12/01, [redacted], [redacted]; 2006**
Title:	Non-target terrestrial plants: an evaluation of the effects of BYI 08330 OD 150 in the vegetative vigour test (Tier 1). Date: 2006-06-20
Organisation:	Bayer CropScience GmbH, Ecotoxicology, Frankfurt
Report No.:	VV06/03-Tier 1; M-273381-01-1
Publication:	unpublished
Dates of experimental work:	March 28, 2006 - April 20, 2006
Guidelines:	OECD 208 B (July 2000, draft): vegetative vigour test (Tier 1)
Deviations:	None
GLP:	No

Executive summary

The aim of the study was to determine phytotoxic effects of BYI 08330 OD 150 on non-target terrestrial plant species in a vegetative vigour test following a post emergence application of the product onto the foliage of plants at the 2-to 4 -leaf growth stage.

Six species of terrestrial non-target plants (2 monocotyledonous and 4 dicotyledonous) were treated at an application of 288 g a.s./ha BYI 08330 OD 150. The species tested were corn (*Zea mays*), oats (*Avena sativa*), cucumber (*Cucumis sativus*), oilseed rape (*Brassica napus*), soybean (*Glycine max*), and sunflower (*Helianthus annuus* L.). Plants were treated at the 2-4-leaf stage with foliar spray application. Spray treatments were applied once, at test initiation, with a sprayer set at the nominal spray volume of 200 litres/ha. Control pots were sprayed with deionised water. Four to five replicates with four to five plants per pot for each species were tested. All pots were individually contained in saucers and retained on benches within a greenhouse. Plants were assessed for survival and phytotoxicity on days 7, 14 and 21. At study termination, endpoint determinations were performed for plant dry weights.

Several significant adverse effects (i.e. greater than 50%) were found after an application of 288 g a.s./ha BYI 08330 OD 150 to representative non-target crops in the vegetative vigour test.

Material and Methods

A Materials

1. Test material	**Spirotetramat (BYI 08330) 150 OD**
Description	**Light brown suspension**
Lot/batch No.	**Batch no.: 08030/0233(0152)**
	Tox no.: TOX07087-00
Analytical content	**148.28 g a.s./L**
Stability of test compound	**Not specified**
2. Vehicle and/or positive control	**Deionised water**
3. Test organisms	

Species	**2 monocotyledonous plant species: corn (*Zea mays*), oats (*Avena sativa*) 4 dicotyledonous plant species: cucumber (*Cucumis sativus*), oilseed rape (*Brassica napus*), soybean (*Glycine max*), sunflower (*Helianthus annuus* L.)**
Plant growth stage at application	**2 to 4 -leaf stage**
Source	**Seeds were supplied from commercial sources via Bayer CropScience GmbH, Horticulture, Frankfurt am Main.**
Acclimation	**Glasshouse conditions Bayer CropScience GmbH**
Environmental conditions	
Temperature	**23 ± 5°C day 18 ± 5°C night**
Photoperiod	**16:8 hours (Natural daylight supplemented by artificial lighting to provide required photoperiod. at >10000 lux lamps turn off, at >20000 lux shading closing.)**

B Study design and methods

1. In life dates **March 28, 2006 - April 20, 2006**

2. Experimental treatments

Seeds used on the study had not been treated with pesticides or repellents prior to test initiation. Routine germination tests were carried out on the seeds to ensure their viability. Seeds were stored in plastic box in refrigerator.

Plants are grown from seed to the 2 to 4-leaf stage in commercial plastic flower pots, 13 cm diameter, containing 950 g soil. Standard soil was used (sterilized with 120 degrees vapour for about 30 minutes and fertilized with 2.4 g Blaukorn per L) composed of 14.2% sand, 65.1% silt and 20.7% clay; pH 7.4 and 1.19% organic carbon. 1 mL Wuxal super solution as a fertilizer was applied on test days 7 and 14. The test item was sprayed on the leaves and above-ground portions of the plants. 4-5 pots per treatment group were sprayed depending on the species with 4-5 plants per pot, also depending on the species. Exposure time was 21 days.

The blank control spray solution was deionised water. The test item was dissolved in deionised water and was applied once at a nominal concentration of 288 g a.s./ha.

A daily spot check was carried out concerning soil moisture.

Bottom watering was performed via saucers standing below each pot. Water was given in the saucer according to the need of the plants in order to have an optimal water supply for plant growth. Temperature and humidity were recorded with thermo-hygrographs.

3. Observations

Mortality/Survival: **Dead plants were removed after each assessment. Number of plants that survived after application was recorded at the final assessment (day 21).**

Phytotoxicity records: **Visual phytotoxicity ratings were recorded (e.g. chlorosis, necrosis, stunting, abnormal growth) at days 7, 14 and 21 according to EPPO Standard 135.**

Growth stages: **Growth stages at day 21 were recorded according to BBCH-Monograph - Growth stages.**

Plant biomass: **The dry weights were determined at the final assessment (day 21). The plants of one pot represent one replicate.**

RESULTS AND DISCUSSION

A. Findings

A summary of all the assessments for the day 21 vegetative vigour test (Tier 1) for the effects of BYI 08330 OD 150 are shown in the table below:

	Cucumber	Oilseed rape	Soybean	Sunflower	Corn	Oats
Survival * (% of control)	5	0	0	0	0	0
Phytotoxicity (see below)	B-C	D	A	B-C	C	B
Dry Weight ** (% inhibition)	41.3	83.3	40.2	43.1	56.9	56.6

* Survival is a measure of treated plants that survived and is expressed as an inhibition of the control.
** inhibition expressed on a per plant basis (figures in parentheses indicate that there was an increase in dry weight when compared to the control)
phytotoxicity:
0 = no injury or effect
A = slight symptom(s) throughout the whole plant or more moderate symptom(s) on a small area, i.e. one leaf
B = moderate symptom(s) throughout the whole plant or severe symptom(s) on a limited area, i.e. one-two leaves
C = severe symptom(s) throughout the whole plant with younger or newly developed leaves growing normally
D = total plant symptom(s) with the plant showing poor vigour.
E = moribund

B. Observations

There were no adverse effects of Spirotetramat OD 150 on the survival of the six species tested.

There were visible signs of phytotoxicity such as chlorosis, necrosis, leaf deformation, bleaching, and/or stunting in all crops tested. There were differences in growth stages in corn and oats.

Biomass was reduced in all species tested. These differences were significant at the 95% confidence limits (Statistical analysis was carried out using the Pairwise Mann-Whitney-U-test, one sided smaller).
The differences exceeded the 50% trigger in three of the species tested.

CONCLUSION

An application rate of 288 g a.s./ha BYI 08330 OD 150 showed several significant adverse effect (i.e. greater than 50%) to representative non-target crops in the vegetative vigour test. These results trigger further testing.

Report:	**KIIA 8.12/02, [redacted], [redacted], 2006**
Title:	Tier I and Tier II Vegetative Vigor Non-Target Plant Study using BYI 08330 150 OD.
	Date: 2006-08-09
Organisation:	Bayer CropScience, Ecotoxicology, South Metcalf, Stilwell, Kansas
Report No.:	EBFNX016, M-276131-01-1
Publication:	unpublished
Dates of experimental work:	November 29, 2005 - December 30, 2005
Guidelines:	Subdivision J, Guidelines 122-1 & 123-1 OECD 208 (draft)
Deviations:	none
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to assess phytotoxic effects during the vegetative growth stages following a post-emergent foliar application of the end-use product, BYI 08330 150 OD, on ten non-target terrestrial plant species over a 21 day period. Ten species of non-target terrestrial plants (4 monocotyledonous and 6 dicotyledonous) were sprayed at various application rates of the formulation. The monocot species oat, onion, corn and ryegrass were treated at application rates of 0 control, 11, 22, 44, 88, 176 g a.s./ha and the dicot species canola, cucumber, soybean, tomato, sugarbeet and sunflower were treated at application rates of 0 (control) and 176 g a.s./ha. All plants were at the two to four leaf stage on the day of application and test duration was 21 days following spray application of the test substance. Spray treatments were applied once to each species at test initiation with an Allen Track sprayer set at the nominal spray volume of 10 gallons/acre or 93.5 L/ha. Control pots were sprayed with deionised water. Spray stock solutions of each treatment were analysed for the quantification of BYI 08330 with a recovery range of 104 to 114% of nominal. Eight replicates with five plants per replicate for each species were tested in natural topsoil. All pots were randomized on benches within a greenhouse. Plants were assessed for plant survival, phytotoxicity, seedling length, and dry weight.

The most sensitive endpoint was corn dry weight with an EC_{25} value of 76 g a.s./ha and an EC_{50} value of 134 g a.s./ha. The Lowest Observed Effect Concentration (LOEC) for corn was 88 g a.s./ha. The No Observed Effect Concentration (NOEC) for corn was 44 g a.s./ha.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) 150 OD**
Description	**Brown liquid**
Lot/batch No.	**Batch no.: 08030/0189(0152)**
Analytical content	**15.1% w/w**
Stability of test compound	**Not specified**
2. Vehicle and/or positive control	**Deionised water**
3. Test organisms	
Species	**4 monocotyledonous plant species from 3 different families:** **corn (*Zea mays*, Poaceae), oat (*Avena sativa*, Gramineae), ryegrass (*Lolium perenne*, Poaceae), onion (*Allium cepa*, Alliaceae)** **6 dicotyledonous plant species from 6 different families:** **cucumber (*Cucumis sativus*, Cucurbitaceae), canola (*Brassica napus*, Brassicaceae), soybean (*Glycine max.*), sunflower (*Helianthus annuus* L., Asteraceae), sugarbeet (*Beta vulgaris*, Chenopodiaceae), tomato (*Lycopersicon esculentum*, Solanaceae)**
Plant growth stage at application	**2 to 4-leaf stage**
Source	**Source, variety and historical germination of the seeds are given in the report. Seeds were not treated with fungicides, insecticides, or repellents prior to test initiation. Seeds were stored under dark conditions in a freezer prior to use.**
Acclimation	**Greenhouse conditions**
Environmental conditions	
Temperature	**18.6 to 22.5°C (humidity 37.1 to 61.2%)**
Photoperiod	**16:8 hours light/dark**
	Light level (log lumens per ft^2): 1.0 to 1.3

B Study design and methods

1. In life dates	**November 29, 2005 - December 30, 2005**
2. Experimental treatments	

Plants are grown from seed to the 2 to 4-leaf stage in plastic pots, 12.7 cm diameter x 10.2 cm depth with bottom drainage holes. Seeds were planted to a depth of approximately 2.5 cm for cucumber, corn, oat, ryegrass, sunflower, and soybean. Canola, lettuce, onion, and tomato were planted to a depth of approximately 1.3 cm. All pots contained five plants following thinning. Tap water was delivered to vinyl dishes containing the pots. Capillary watering occurred approx. once per day.

The soil (silt loam) used for this study was a mixture of a natural topsoil and sand collected from Johnson County Top Soil and Landscape Materials, Stilwell, KS., characterized by 28% sand, 50% silt and 22% clay, 2.4% organic carbon, cation exchange capacity [meq/100 g] = 15.5 and pH 7.8. Osmocote™ slow-release fertilizer was added to the soil prior to use.

Each crop was allowed to grow to approximately the 2-4 true leaf stage prior to spray application. The spray nozzle was positioned 33.02 cm (= 13 inches) above the plant canopy for each species. The spray application was performed using an Allen Track Sprayer (Allen Machine Works, Midland, MI). The track sprayer was equipped with a pneumatically driven spray boom and a Tee-Jet flat-fan spray nozzle and was calibrated to simulate the application rate of 93.5 L/ha. Based on a non-GLP range finding test, the application rates were 0 (control), 11, 22, 44, 88, 176 g a.s./ha for the monocot species oat, onion, corn and ryegrass and 0 (control) and 176 g a.s./ha for the dicot species canola, cucumber, soybean, tomato, sugarbeet and sunflower. 176 g a.s./ha is the highest single application planned for the United States.

3. Observations

Samples of the spray stock solutions were collected at study initiation on Day 0 and analysed for the active substance spirotetramat.

The test endpoints included plant survival, shoot length and dry weight. Plant survival and phytotoxic effects were determined weekly (i.e. Days 7, 14, and 21) by visual enumeration using the following rating system: 0 (no injury/effect), 10 to 20% (indicates slight effect), 30 to 40% (indicates moderate effect), 50 to 60% (indicates severe effect), 70 to 80% (indicates total plant effect), 90% and above (plant death).

On Day 21 plants were excised at the soil surface and shoot heights were measured to the longest leaf for each plant. Following plant length measurements, all plants placed in a drying oven for approximately 48 hours at 70ºC. Replicate dry weight measurements were determined to the nearest 0.001 g using an electronic balance. For each test, descriptive statistics (mean and standard deviation) and percent effect values were calculated for each treatment against the controls. The replicate means were used in all statistical analyses. Descriptive statistics were performed using EXCEL. Emergence and survival were calculated by the probit method. The NOEC/LOEC values were identified using hypothesis testing methodology. All Day 21 data were subjected to a Shapiro-Wilk's Test to assess departures from a normal distribution and a Levene's Test to determine homogeneity of variance. To assess treatment effects for homoscedastic and normally distributed data, a Dunnett's one-

way analysis of variance (ANOVA) and multiple means comparison procedure for equal replicates was used to determine those exposure concentrations exhibiting responses significantly different ($p \leq 0.05$) than the control group. Data not meeting assumptions of normality or homogeneity of variances were transformed by various methods. If data transformation methods were unsuccessful, the data were subjected to a non-parametric statistical method. All statistical analyses were performed using the computer program SAS Procedure NLIN (Version 6.12).

RESULTS AND DISCUSSION

A. Findings

A summary of the Day 21 No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC), EC_{25} and EC_{50} and values for each of the plant species obtained in the vegetative vigour test (Tier 1) for the effects of BYI 08330 OD 150 is given the following tables:

Vegetative Vigor Survival						
Species	EC_{25} [g a.s./ha]	± 95% Confidence Limits	EC_{50} [g a.s./ha]	± 95% Confidence Limits	LOEC [g a.s./ha]	NOEC [g a.s./ha]
Canola	>176	n.a.	>176	n.a.	>176	176
Corn	>176	n.a.	>176	n.a.	>176	176
Cucumber	>176	n.a.	>176	n.a.	>176	176
Oat	>176	n.a.	>176	n.a.	>176	176
Onion	>176	n.a.	>176	n.a.	>176	176
Ryegrass	>176	n.a.	>176	n.a.	>176	176
Soybean	>176	n.a.	>176	n.a.	>176	176
Sugarbeet	>176	n.a.	>176	n.a.	>176	176
Sunflower	>176	n.a.	>176	n.a.	>176	176
Tomato	>176	n.a.	>176	n.a.	>176	176

n.a. = not applicable

Vegetative Vigor Plant Length						
Species	EC_{25} [g a.s./ha]	± 95% Confidence Limits	EC_{50} [g a.s./ha]	± 95% Confidence Limits	LOEC [g a.s./ha]	NOEC [g a.s./ha]
Canola	>176	n.a.	>176	n.a.	>176	176
Corn	106	92 to 122	172	157 to 189	88	44
Cucumber	>176	n.a.	>176	n.a.	>176	176
Oat	>176	n.a.	>176	n.a.	>176	176
Onion	>176	n.a.	>176	n.a.	>176	176
Ryegrass	>176	n.a.	>176	n.a.	176	88
Soybean	>176	n.a.	>176	n.a.	>176	176
Sugarbeet	>176	n.a.	>176	n.a.	>176	176
Sunflower	>176	n.a.	>176	n.a.	>176	176
Tomato	>176	n.a.	>176	n.a.	>176	176

n.a. = not applicable

Vegetative Vigor Plant Dry Weight						
Species	EC_{25} [g a.s./ha]	± 95% Confidence Limits	EC_{50} [g a.s./ha]	± 95% Confidence Limits	LOEC [g a.s./ha]	NOEC [g a.s./ha]
Canola	>176	n.a.	>176	n.a.	>176	176
Corn	76	60 to 96	134	117 to 155	88	44
Cucumber	>176	n.a.	>176	n.a.	>176	176
Oat	>176	n.a.	>176	n.a.	>176	176
Onion	>176	n.a.	>176	n.a.	>176	176
Ryegrass	168	153 to 184	>176	n.a.	176	88
Soybean	>176	n.a.	>176	n.a.	>176	176
Sugarbeet	>176	n.a.	>176	n.a.	>176	176
Sunflower	>176	n.a.	>176	n.a.	>176	176
Tomato	>176	n.a.	>176	n.a.	>176	176

n.a. = not applicable

B. Observations

Canola: Plant survival was not significantly affected at the 176 g a.s./ha treatment level in comparison to the controls. Phytotoxicity was observed in one replicate on Day 21 with minor observations of stunting. Plants were not significantly different for plant dry weight or length at the 176 g a.s./ha treatment by Dunnett's Test as compared to the controls.

Corn: Plant survival was not significantly affected at any treatment level in comparison to the controls. Phytotoxicity was observed throughout the study with observations of stunting, bleaching, leaf curling, and necrosis with severity of effects increasing with treatment concentration. Plants were significantly different for plant length and dry weight at the 88 and 176 g a.s./ha treatments by Dunnett's Test as compared to the controls. The EC_{25} dry weight

values were determined as 76 g a.s./ha by non-linear regression, respectively.
Oat: Plant survival was not significantly affected at any treatment level in comparison to the controls. Random phytotoxicity was observed throughout the study with observations of stunting, bleaching, leaf curling, and necrosis with severity of effects lessening by Day 21 in the higher treatment rates. Plants were not significantly different for plant length and dry weight for any treatment by Dunnett's Test as compared to the controls.
Onion: Plant survival was not significantly affected at any treatment level in comparison to the controls. Minor phytotoxicity was observed throughout the study with observations of stunting, leaf curling, and necrosis. Plants were not significantly different for plant length and dry weight for any treatment by Dunnett's Test as compared to the controls.
Ryegrass: Plant survival was not significantly affected at any treatment level in comparison to the controls. Phytotoxicity was observed throughout the study with observations of stunting, bleaching, leaf curling, and necrosis with severity of effects increasing with treatment concentration. Plants were not significantly different for plant length. Plants were significantly different for plant dry weight at the 176 g a.s./ha treatment by Dunnett's Test as compared to the controls. The EC_{25} dry weight values were determined as 168 g a.s./ha.
Cucumber, soybean, sugarbeet, sunflower, tomato: Plant survival was not significantly affected at the 176 g a.s./ha treatment level in comparison to the controls. No phytotoxicity was observed in any treatment. Plants were not significantly different for plant dry weight or length at the 176 g a.s./ha treatment by Dunnett's Test as compared to the controls. The EC_{25} plant length and dry weight values were determined as >176 g a.s./ha.

CONCLUSION

In a vegetative vigour study with 10 non-target terrestrial plant species, following a 21 day post-emergent foliar application of BYI 08330 150 OD, the most sensitive endpoint was corn dry weight with an EC_{25} value of 76 g a.s./ha and an EC_{50} value of 134 g a.s./ha. The Lowest Observed Effect Concentration (LOEC) for corn was 88 g a.s./ha. The No Observed Effect Concentration (NOEC) for corn was 44 g a.s./ha.

Report:	**KIIA 8.12/03, [redacted]; 2006**
Title:	Spirotetramat OD 150: Vegetative vigour test on non-target terrestrial plants Date: 2006-10-30
Organisation:	Source: BioChem agrar GmbH, Gerichshain, Germany, Owner: Bayer CropScience, Germany
Report No.:	061048126; M-279698-01-1
Publication:	unpublished
Dates of experimental work:	July 07, 2006 – August 04, 2006
Guidelines:	OECD 227 (Draft March 2005): Vegetative Vigour Test
Deviations:	None
GLP:	Yes

Material and methods

Test item: Spirotetramat OD 150, containing: BYI 08330 (Spirotetramat) 150 g/L (15.1% w/w analysed), Specification No.: 102000011380, Batch No.: 08030/0189(0152), Sample description: FAR01219-00, density (20°C): 0.991 g/mL; control: water treated.

In three experiments, each with a duration of 21 days, the potential effects of the test item Spirotetramat OD 150 to 3 plant species (*Brassica napus, Avena sativa, Zea mays*) was examined in comparison with control treatments under greenhouse conditions.

In the experiments, the test item Spirotetramat OD 150 was applied to the test plants at nominal application rates of 0.17, 0.48, 0.96, 1.92 L product/ha (oilseed rape, oats, corn) in 400 L/ha water. Plants in the 2-leaf stage were used. The test item was sprayed onto the plant foliage. The recovery rate of the analysed highest dosed test solutions ranged from 82.1 – 90.9% (corrected for the recovery rate of validation). Survival (mortality), shoot fresh weight, visual phytotoxicity and growth inhibition of the plants were recorded and treated plants were evaluated against untreated controls for inhibitory effects. Statistical analysis of data was performed to obtain NOEC values and EC_{50}/LC_{50}, using the software ToxRat Professional 2.09 (RATTE 2005). The following results were obtained for survival and shoot fresh weight at the final assessment on day 21 after application.

Findings

The results were summarised in the following table:

21 days after application	**Spirotetramat OD 150** (L product/ha in 400 L/ha)			
Plant species	**Survival**	**Shoot fresh weight**		BBCH
	LC_{50}	**EC_{50}**	**NOEC**	
Brassica napus	> 1.92	> 1.92	0.48	15-17
Avena sativa	> 1.92	> 1.92	0.96	16-21
Zea mays	1.92	1.92	0.96	16-18

Observations:

Brassica napus:

The foliar application of Spirotetramat OD 150 onto oilseed rape plants had no effect on survival up to the highest application rate of 1.92 L product/ha.

Growth in terms of fresh weight on day 21 after application was significantly reduced starting with the tested application rate of 0.96 L product/ha. The NOEC with respect to growth inhibition was 0.48 L product/ha. The 21 d EC_{50} value for fresh weight was determined to be > 1.92 L product/ha.

No necrotic and chlorotic symptoms were observed on day 21 at all rates tested. Growth inhibitory effects were observed for the two highest rates of 0.96 and 1.92 L product/ha.

Avena sativa
Application of Spirotetramat OD 150 to oat plants caused no lethal effects up to the highest application rate of 1.92 L product/ha.
Regarding fresh weight decrease, a NOEC of 0.96 L product/ha was calculated. The 21 d EC_{50} value for fresh weight was determined to be > 1.92 L product/ha.
No necrotic and chlorotic symptoms were observed on day 21 at all rates tested. Growth inhibitory effects were observed for the two highest rates of 0.96 and 1.92 L product/ha.

Zea mays
The foliar application of Spirotetramat OD 150 onto corn plants had no effect on survival up to the highest application rate of 1.92 L product/ha.
Growth in terms of fresh weight on day 21 after application was significantly reduced starting with the tested application rate of 1.92 L product/ha. The NOEC with respect to growth inhibition was 0.96 L product/ha. The 21 d EC_{50} value for fresh weight was determined to be > 1.92 L product/ha.
Necrotic, chlorotic and growth inhibitory effects were observed for the two highest rates of 0.96 and 1.92 L product/ha.

Conclusion
A vegetative vigour test with Spirotetramat OD 150 on *Brassica napus, Avena sativa* and *Zea mays* resulted in an EC_{50} of > 1.92 L product/ha (corresponding to > 288 g a.s./ha).

Report:	**KIIIA1 8.12/04, [redacted], [redacted].; (2006)**
Title:	Non-target terrestrial plants: an evaluation of the effects of BYI 08330 OD 150 in the seedling emergence and growth test (Tier 1). Date: 2006-06-20
Organisation:	Bayer CropScience GmbH, Ecotoxicology, Frankfurt
Report No.:	SE06/03 Tier 1; M-273373-01-1
Publication:	unpublished
Dates of experimental work:	March 28, 2006 - April 18, 2006
Guidelines:	OECD 208 A (July 2000, draft): seedling emergence and growth test (Tier 1)
Deviations:	None
GLP:	No

Executive summary

The aim of the study was to determine phytotoxic effects of BYI 08330 OD 150 on non-target terrestrial plant species in the seedling emergence and growth test following a pre-emergence application of the product.
Six species of terrestrial non-target plants (2 monocotyledonous and 4 dicotyledonous) were treated at an application of 288 g a.s./ha BYI 08330 OD 150, i.e. the highest recommended application rate planned for Europe. The species tested were corn (*Zea mays*), oats (*Avena sativa*), cucumber (*Cucumis sativus*), oilseed rape (*Brassica napus*), soybean (*Glycine max*), and sunflower (*Helianthus annuus* L.).
All seeds were planted on the day of application and test duration was 14 days after 70% emergence of the seedling in the controls for each species.
Spray treatments were applied once, at test initiation, with a sprayer set at the nominal spray volume of 200 litres/ha. Control pots were sprayed with deionised water. Four replicates with five seeds per pot for each species were tested. All pots were individually contained in saucers and retained on benches within a greenhouse. Plants were assessed for emergence, survival and rated for phytotoxicity on days 7 and 14. At study termination, biomass endpoint determinations were performed for plant dry weights.
No significant adverse effect (i.e. greater than 30%) were found after an application of 288 g a.s./ha BYI 08330 OD 150 to representative non-target crops in the seedling emergence and growth test.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) 150 OD**
Description	**Light brown suspension**
Lot/batch No.	**Batch no.: 08030/0233(0152)** **Tox no.: TOX07087-00**
Analytical content	**148.28 g a.s./L**
Stability of test compound	**Not specified**
2. Vehicle and/or positive control	**Deionised water**
3. Test organisms	
Species	**2 monocotyledonous plant species: corn (*Zea mays*), oats (*Avena sativa*)** **4 dicotyledonous plant species: cucumber (*Cucumis sativus*), oilseed rape (*Brassica napus*), soybean (*Glycine max*), sunflower (*Helianthus annuus* L.)**
Plant growth stage at application	**Seeds in the soil**
Source	**Seeds were supplied from commercial sources via Bayer CropScience GmbH, Horticulture, Frankfurt am Main.**
Acclimation	**Glasshouse conditions Bayer CropScience GmbH**

Environmental conditions	
Temperature	**23 ± 5°C day**
	18 ± 5°C night
Photoperiod	**16:8 hours**
	(Natural daylight supplemented by artificial lighting to provide required photoperiod at >15000 lux lamps turn off, at >20000 lux shading closing.)

B Study design and methods

1. In life dates **March 28, 2006 - April 18, 2006**

2. Experimental treatments

Seeds used on the study had not been treated with pesticides or repellents prior to test initiation. Routine germination tests were carried out on the seeds to ensure their viability. Seeds were stored in plastic box in refrigerator.

The day before application the seeds were introduced manually in the soil. They were covered by about there own size (2 - 5 mm) with soil. After sowing the pots were be top watered.

Plants are grown from seed to the 2 to 4-leaf stage in commercial plastic flower pots, 13 cm diameter, containing 950 g soil. Standard soil was used (sterilized with 120 degrees vapour for about 30 minutes and fertilized with 2.4 g Blaukorn per L) composed of 14.2% sand, 65.1% silt and 20.7% clay, pH 7.4 and 1.19% organic carbon. No further addition of nutrient medium to the plants was necessary in the study.

The spray solution was applied to the soil surface. 4 pots per treatment group were sprayed, containing 5 seeds per pot. Exposure time was 14 days after 70% emergence of the seedling in the controls for each species.

The blank control spray solution was deionised water. The test item was dissolved in deionised water and was applied once at a nominal concentration of 288 g a.s./ha. After sowing into the soil initial top watering was carried out to stimulate germination. Then bottom watering was performed via saucers standing below each pot. Water was given in the saucer according to the need of the plants in order to have an optimal water supply for plant growth. A daily spot check was carried out concerning soil moisture.

3. Observations

<u>Germination:</u> Daily checks were made to identify the date when 70% of the seedlings emerge in the control for each species. Number of plants was counted after 7 and 14 days.

<u>Mortality/Survival:</u> Dead plants were removed after each assessment. Number of plants that survived after application was recorded at the final assessment.

<u>Phytotoxicity records:</u> Visual phytotoxicity ratings were recorded (e.g. chlorosis, necrosis, stunting, abnormal growth) 7 and 14 days after the emergence of 70% of seeds in the controls. Phytotoxicity was assessed according to EPPO Standard 135.

Growth stages: **Growth stages were recorded at the final assessment according to BBCH-Monograph - Growth stages.**

Plant biomass: **The dry weights were determined at the final assessment (day 14 after 70% emergence in the untreated control). The plants of one pot represent one replicate.**

RESULTS AND DISCUSSION

A. Findings

A summary of all the assessments at the end of the seedling emergence and growth test (Tier 1) for the effects of BYI 08330 OD 150 are shown in the table below:

	Cucumber	Oilseed rape	Soybean	Sunflower	Corn	Oats
Germination (% inhibition)	(11.1)	0	0	(5.3)	0	0
Survival * (% of control)	0	0	0	0	5	0
Phytotoxicity (see below)	0	0	0	0	0	0-A
Dry Weight ** (% inhibition)	(0.9)	(8.1)	0.2	(1.1)	10.6	8.1

* Survival is a measure of treated plants that survived and is expressed as an inhibition of the control.

** inhibition expressed on a per plant basis

figures in parentheses indicate that there was an increase in dry weight when compared to the control

phytotoxicity: 0 = no injury or effect

A = slight symptom(s) throughout the whole plant or more moderate symptom(s) on a small area, i.e. one leaf

B. Observations

Statistical analysis was carried out using the Pairwise Mann-Whitney-U-test (one sided smaller).

All emerged control plants exhibited normal growth.

There was no significant adverse effect of BYI 08330 OD 150 on the six species tested. Biomass was less compared to control in soybean, corn and oats by 0.2%, 10.6% and 8.1%, respectively. Biomass was greater compared to control in cucumber, oilseed rape and sunflower by 0.9%, 8.1%, and 1.1% respectively. None of these differences were significant at the 95% confidence limits and no adverse effect reached or exceeded the 50% trigger for further testing.

CONCLUSION

An application of 288 g a.s./ha BYI 08330 OD 150 showed no significant adverse effect (i.e. greater than 50%) to representative non-target crops in the seedling emergence and growth test.

Report:	**KIIA 8.12/05, [redacted]., [redacted].; 2006**
Title:	Tier I Seedling Emergence Non-Target Plant Study using BYI 08330 150OD. Date: 2006-08-09
Organisation:	Bayer CropScience, Ecotoxicology, South Metcalf, Stilwell, Kansas
Report No.:	EBFNX017; M-276244-01-1
Publication:	unpublished
Dates of experimental work:	November 23, 2005 - December 14, 2005
Guidelines:	Subdivision J, Guideline 122-1
Deviations:	no major deviations
GLP:	yes (certified laboratory)

Executive summary

The phytotoxicity of BYI 08330 150OD on ten non-target plant species was evaluated in a seedling emergence study. The formulation was applied on the soil surface at the maximum single application rate of 176 g a.s./ha in the U.S. A control consisting of deionised water (i.e. the carrier) was included in the study design. The non-target terrestrial plants which were evaluated in a greenhouse consisted of four monocots (corn, onion, rye grass and oat), and six dicots (sugarbeet, canola, cucumber, sunflower, soybean, and tomato). The treatment rate was applied at approximately 93.5 L/ha or 10 GPA (gallon per acre). Endpoints included seedling emergence, phytotoxicity, plant survival, shoot height and dry weight.

No significant adverse effect for any measured parameter at or above 25% was found as compared to the controls in this Tier I seedling emergence study.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) 150 OD**
Description	**Brown liquid**
Lot/batch No.	**Batch no.: 08030/0189(0152)**
Analytical content	**15.1% w/w**
Stability of test compound	**Not specified**
2. Vehicle and/or positive control	**Deionised water**
3. Test organisms	
Species	**4 monocotyledonous plant species from 3 different families:** **corn (*Zea mays,* Poaceae), oat (*Avena sativa,* Gramineae), ryegrass (*Lolium perenne*, Poaceae), onion (*Allium cepa*, Alliaceae)** **6 dicotyledonous plant species from 6 different families:** **cucumber (*Cucumis sativus,* Cucurbitaceae),**

	canola (*Brassica napus,* Brassicaceae), soybean (*Glycine max.*), sunflower (*Helianthus annuus* L., Asteraceae), sugarbeet (*Beta vulgaris*, Chenopodiaceae), tomato (*Lycopersicon esculentum*, Solanaceae)
Plant growth stage at application	**Seeds in the soil**
Source	**Source, variety and historical germination of the seeds are given in the report. Seeds were not treated with fungicides, insecticides, or repellents prior to test initiation. Seeds were stored under dark conditions in a freezer prior to use.**
Acclimation	**Greenhouse conditions**
Environmental conditions	
Temperature	**18.6 to 24.4°C (humidity 27.6 to 70.6%)**
Photoperiod	**16:8 hours light/dark**
	Light level (log lumens per ft²): 0.8 to 1.3

B Study design and methods

1. In life dates **November 23, 2005 - December 01, 2005**

2. Experimental treatments

The soil (silt loam) used for this study was a mixture of a natural topsoil and sand collected from Johnson County Top Soil and Landscape Materials, Stilwell, KS., characterized by 28% sand, 50% silt and 22% clay, 2.4% organic carbon, cation exchange capacity [meq/100 g] = 15.5 and pH 7.8. The soil was steam pasteurized at Bayer CropScience and Osmocote™ slow-release fertilizer was added to the soil prior to use.

Test seeds were planted in plastic pots (12.7 cm diameter x 10.2 cm depth) with bottom drainage holes. Seeds of approximately the same size were planted to a depth of 2.5 cm for soybean, cucumber, oat and corn, and 1.3 cm for canola, sunflower, sugarbeet, tomato, ryegrass and onion. Each pot contained five seeds per pot. Eight replicates (e.g. pots) were tested for each treatment concentration and control. The seeds for the seedling emergence study were planted the day prior to spray application. All test pots were contained in vinyl dishes and placed on greenhouse benches. Pots were bottom watered following spray application to prevent wetting of the foliage. Dishes were checked twice daily and were watered on average once per day by adding tap water to each dish. The spray solution was applied to the soil surface using an Allen Track Sprayer. The concentration tested was 176 g a.s./ha. This is the highest single application planned for the United States.

3. Observations

Samples of the spray stock solutions were collected at study initiation on Day 0 and analysed for the active substance spirotetramat.

The endpoints evaluated on these study days were the number of emerged seedlings, number of seedlings surviving, and phytotoxicity of each replicate and were evaluated on Day 7, Day 14, and Day 21 following spray application by visual enumeration using the following rating system: 0 (no injury/effect), 10 to 20% (indicates slight effect), 30 to 40% (indicates moderate effect), 50 to 60% (indicates severe effect), 70 to 80% (indicates total plant effect), 90% and above (plant death). Plants were excised at the soil surface on Day 21 for plant height and dry weight determination. Plant shoot height was measured by extending cut seedlings to their maximum length and recording. Plant dry weight was performed by placing all replicate plants within labelled paper bags. The plants were placed in a drying oven and allowed to dry for at least 48 hours at approximately 70ºC. Plant dry weight measurements were determined to the nearest 0.001 g using an electronic balance. All Day 21 data were subjected to a Shapiro-Wilk's Test to assess departures from a normal distribution and a Levene's Test to determine homogeneity of variance. To assess treatment effects between the control and treatment group, a Student's t-test was performed. All statistical analyses were performed using the computer program SAS, descriptive statistics were performed using EXCEL.

RESULTS AND DISCUSSION

A. Findings

The nominal BYI 08330 concentrations for the study were 0 (control) and 176 g a.s./ha (1882 mg a.s./L testing solution). The measured BYI 08330 concentration in the seedling emergence study was 1954 mg/L representing 104% of nominal. There were no BYI 08330 residues found in the control samples. All reported values were based on the nominal amount of the application rate.

The photoperiod in the study deviated from the protocol range of 16 hours light: 8 hours dark. The photoperiod deviated for a few days ranging from 13 to 18 hours light for duration of the study. The deviation was minor and does not impact the results of the study.

Percent seedling emergence for the 10 crop species ranged from 88 to 100% in the controls, and 88 to 100% in the 176 g a.s./ha treatment. Percent seedling survival for the 10 crop species ranged from 91 to 100% in the controls, and 84 to 100% in the 176 g a.s./ha treatment.

Phytotoxicity ratings for the seedling emergence test ranged from 1 to 4% for the 176 g a.s./ha treatment for all species. Similar phytotoxicity of minor necrosis and stunting was observed in the controls and treated levels of cabbage, cucumber, and turnip. The observed phytotoxicity was considered to be background as the level of incidence was similar in both the control and treated levels.

Percent inhibition for plant length for the 10 crop species ranged from -4 to 8% in comparison to the controls and the 176 g a.s./ha treatment. Percent inhibition for plant dry weight for the 10 crop species ranged from -13 to 19% in comparison to the controls and the 176 g a.s./ha treatment.

The percent effect for each end-point (i.e. emergence, survival, plant length, dry weight) was 19% or less for each crop species. No significant effects were observed to any of the 10 plant species tested.

Summary of seedling emergence and survival

	21 d seedling emergence		21 d seedling survival	
Treatment	Control	176 g a.s./ha	Control	176 g a.s./ha
Species	% Emergence	% Emergence	% Survival	% Survival
Canola	88	88	91	84
Corn	93	98	100	100
Cucumber	95	98	97	100
Oat	98	98	100	100
Onion	100	98	100	100
Ryegrass	100	95	100	100
Soybean	98	98	100	100
Sugarbeet	93	95	100	97
Sunflower	95	100	100	100
Tomato	90	90	100	100

% Emergence = (No. plants emerged / No. seeds planted) x 100
% Survival = (No. plants surviving / No. seeds emerged) x 100

CONCLUSION

There were no significant adverse effects for any measured parameter at or above 25% as compared to the controls in a Tier I seedling emergence study at the single highest application rate of 176 g a.s./ha planned for the United States. Because there were no effects at or above 25%, a Tier 2 study is not required.

IIA 8.13 Effects on terr. vertebrates other than birds / wild mammal toxicity*

Effects on mammals are reported in the toxicology section of this dossier. Risk assessments for wild mammals based on these endpoints are performed in Annex III dossiers for formulated products.

*** No EC data requirement (the OECD point concerned is not covered by or part of an EC point according to Council Directive 91/414/EEC. Hence, data/documents do not need to be submitted.**

IIA 8.14 Effects on other non-target organisms believed to be at risk

Report:	**KIIA 8.14/01, [redacted] & [redacted]; 2005**
Title:	BYI 08330-Enol: Effects on survival and reproduction of the predaceous mite *Hypoaspis aculeifer* Canestrini (Acari: Laelapidae) in standard soil (LUFA 2.1) Date: 2005-01-19
Organisation:	ECT Oekotoxikologie GmbH, Flörsheim, Germany [redacted], Germany
Report No.:	P11HR; M-243307-01-1
Publication:	Unpublished
Dates of experimental work:	November 16, 2004 - December 20, 2004
Guidelines:	SECOFASE, Final Report, Improvement and standardisation of test systems for assessing sub-lethal effects of chemicals on fauna in the soil ecosystem (Løkke & van Gestel 1996). Guidance document on regulatory testing procedures for pesticides with non-target arthropods (Barrett et al. 1994).
Deviations:	None
GLP:	yes (certified laboratory)

Executive summary

The purpose of this study was to determine the LC_{50}, the $NOEC_{Mortality}$ and the $NOEC_{Reproduction}$ for the effects of BYI 08330-enol on the gamasid mite species *Hypoaspis aculeifer* (Isotomidae) by dermal and alimentary uptake using a standard soil (LUFA 2.1).
20 *Hypoaspis aculeifer* (max. 2 days old) were exposed to 10, 32, 100, 316 and 1000 mg pure metabolite/kg dry weight soil (mg p.m./kg d.wt.s.) and water control. The test item was mixed with the substrate.
The study consisted of an exposure period of 14 days to determine mortality and a following period until day 35 to determine mating and reproduction.
Physical-chemical measurements showed that the properties of the test soil were in sufficient agreement with the nominal values.
Since the mortality observed with the test item in the highest concentration was not higher than 42.5%, the LC_{50} value was not calculated and was estimated as being > 1000 mg p.m./kg d.wt.s.
The $NOEC_{Reproduction}$ was determined as ≥ 1000 mg p.m./kg d.wt.s.

MATERIAL AND METHODS

A Materials

1. Test material

Description	**Beige powder**
Lot/batch No.	**Batch no.: 692-101-09-0005** **TOX no.: 06850-00**
Purity	**99.1%**
Stability of test compound	**Stable until 2005-3-17, when stored dry, at temperatures < 40°C**

2. Vehicle and/or positive control	**LUFA 2.1 soil** **Reference item: Perfekthion (5 mg dimethoate/kg)**
3. Test animals	
Species	***Hypoaspis aculeifer* CANESTRINI**
Age	**Protonymphal stage, < 2 days**
Source	**Originally delivered by MITOX Laboratories (Amsterdam, The Netherlands), since February 2002 kept at the ETC**
Acclimation period	**Synchronised culture: six days before the test, adult *H. aculeifer* were transferred to 5 synchronisation units (approx. 180 females and 20 males per unit). Food and water was added. Four days before the start of the test all organisms except eggs were removed. Water was added. Two days later the first protonymphs occurred.**
Environmental conditions	
Temperature	**25 ± 2°C**
Photoperiod	**Permanent dark**

B Study design and methods

1. In life dates **November 16, 2004 - December 20, 2004**

2. Experimental treatments

As artificial test soil, LUFA 2.1, a standard soil obtained from Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer, Germany, was used.

Five concentrations 10, 32, 100, 316 and 1000 mg p.m./kg d.wt.s. and a control were tested, four replicates per concentration and five control replicates. Additionally three replicates were tested with the toxic reference item using one concentration (5 mg dimethoate/kg d.wt.s.).

The study consisted of an exposure period of 14 days to define mortality and a following period until day 35 to define mating and reproduction.

For the mortality exposure, BYI 08330-Enol was mixed in an amount of LUFA 2.1 soil sufficient to prepare a mixture for each test item concentration separately. An appropriate amount of these test item/LUFA 2.1 soil mixtures were incorporated into the remainders of the LUFA 2.1 soil to prepare the different test item concentrations. An appropriate amount of deionised water was added to adjust the final soil moisture of 40 – 60% of WHCmax. At the same time 20 protonymphs were put on the soil surface of each test unit randomly.

Glass containers (able to be closed tightly) of about 30 mL capacity were used for the exposure period (inner diameter of 3.5 cm, height of 4.0 cm). They were filled with 5.1 – 5.3 g f.wt. (fresh weight) (approx. 3 mm layer) of contaminated LUFA 2.1

soil. After the soil substrate was inserted, it was slightly compressed to equalise the surface.

For the mating and reproduction period, plastic containers (able to be closed tightly) of about 12.5 mL with an inner diameter of 2.7 cm and a height of 2.9 cm were used. They were filled with an uncontaminated layer of plaster of Paris (approximately 1.5 cm) darkened with activated carbon. The plaster of Paris was moistened with deionised water until the plaster was almost saturated. Both exposure units were passively ventilated.

On day 14 all surviving adult mites of the untreated water control and of all test item concentrations were transferred to the untreated mating units, keeping replicate groups together.

On day 21 females from the control and the three highest concentrations which caused less than 50% mortality (i.e. 100, 316 and 1000 mg test item/kg d.wt.s.) were transferred individually to the first series of reproduction units (one female per unit) to determine egg/juvenile production. 20 units for the control and the three concentrations were installed. Remaining concentrations (i.e. 10 and 32 mg test item/ d.wt.s.) were discarded. On day 24 females from the first series of reproduction units were transferred individually to a second series of reproduction units (one female per unit), females were removed from the second series of the reproduction units on day 27 and were discarded.

Thrice weekly food was checked and was added on demand. Until day 21 the mites were fed with prey mites (e.g. *Tyrophagus putrescentiae*), then they were fed with enchytraeids (e.g. *Enchytraeus cryptieus*) until the end of the test.

The analytical concentration of the test item was not determined as it is not required according to the methods described in Lokke & van Gestel 1996 and Barrett et al. 1994.

3. Observations

At the start of the study the moisture (weight difference before and after drying) and the maximum water holding capacity ($WHC_{max.}$) of the artificial soil were determined. The pH was measured at the start of the study as well.

During the mortality and mating period the water content of the LUFA 2.1 soil and the plaster of Paris was checked thrice per week by reweighing the test units. Losses of water were compensated. Also the food was checked thrice weekly and prey mites were added.

On day 14, mortality was assessed by counting the number of males/females alive. The number of corpses and the occurrence of offspring (eggs and/or newly hatched juveniles) was recorded additionally.

On day 28, 29 and 30 of the test the number of juveniles in the first series of reproduction units were counted (day 4 - 6 after removal of the females) and on day 32, 33 and 34 of the test the number of juveniles in the second series of reproduction units were counted (day 4 - 6 after removal of the females).

Corrected mortality (mortality due to treatment) was computed according to Abbott (1925). Mortality treatment means were arcsine transformed and data were

checked for normality by R/s test procedure and for homogeneity by Cochran's test. A One-Way Analysis of Variance (ANOVA), followed by a Dunnett's test (1-sided, p ≤ 0.05) was used to determine whether or not there were significant differences in the mean mortality of mites after 14 days between each concentration and the control.

Reproduction data were checked for normality by R/s test procedure and for homogeneity by Cochran's test. The Welch-t test for inhomogeneous variances with Bonferroni Adjustment (1-sided, p ≤ 0.05) was used to determine whether or not there were significant differences in the mean cumulative total number of juveniles (= fertile eggs)/female/7days between the control and the three highest concentrations causing less than 50% mortality. The statistical software package ToxRat Professional 2.09 was used for these calculations.

RESULTS AND DISCUSSION

A. Findings

All validity criteria are met as the mortality/escape rate of the adults in the control after 14 days of exposure was 3% (demanded ≤ 25%); the mean reproduction rate in the control after the 7-day reproduction period was 25.6 ± 7.3 (demanded ≥ 10 fertile eggs (= juveniles) per female) and the mortality/escape (corrected according to Abbott (1925)) rate of the adults in the reference item after 14 days of exposure was 88.0% (demanded 50 – 99.5%).
The temperature ranged from 24 - 25°C, soil pH value was 5.4 - 5.5 and soil moisture was between 54.2% and 60.3% of the WHC_{max}. Only in two cases the observed soil moisture was slightly higher than required. However, this did not influence the test results. All other requirements concerning test performance were fulfilled.
Three percent of adult mites died in the control. At the concentrations of 10, 32, 100 and 316 mg p.m./kg d.wt.s. 5.0 - 15.0% mortality was observed (corresponding to a corrected mortality according to Abbott (1925) from 2.1 to 12.4%). At the highest concentration of the test item tested (1000 mg p.m./kg d.wt.s.) 42.5% mortality was observed (corrected mortality 40.7%). Since the mortality observed with the test item was not higher than 42.5%, the LC_{50} value was not calculated and was estimated as being > 1000 mg p.m./kg d.wt.s.
The ANOVA and the Dunnett's test (1-sided, $p \leq 0.05$) showed a significant difference in the mortality after 14 days between the control and the highest concentration (1000 mg p.m./kg d.wt.s.) of the test item tested. Therefore, the $NOEC_{Mortality}$ was determined as 316 mg p.m./kg d.wt.s. and accordingly the $LOEC_{Mortality}$ was determined as 1000 mg p.m./kg d.wt.s.
Reproduction was examined only for the three highest concentrations of the test item which caused less than 50% mortality (i.e. 100, 316 and 1000 mg p.m./kg d.wt.s.). Statistical analysis (Welch-t test; 1-sided, $p \leq 0.05$) showed no significant difference concerning the cumulative number of juveniles per female after 7 days between the control and these three concentrations of the test item tested. Therefore, the $NOEC_{Reproduction}$ was determined as ≥ 1000 mg p.m./kg d.wt.s.

Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item	BYI 08330-Enol			
Test object	*Hypoaspis aculeifer*			
Exposure	LUFA 2.1 soil, 34 days			
Concentration [mg p.m./kg d.wt.s.]	Average mortality [%]	Corrected mortality [%]	Mean cumulative number of juveniles/female after 7 days [mean ± sd]	Reduction of juveniles [%]
Control	3.0	0	25.6 ± 7.3	0
Reference	88.3	88.0	-	-
10	10.0	7.2	-	-
32	15.0	12.4	-	-
100	5.0	2.1	30.1 ± 3.2	- 17.8
316	10.0	7.2	26.4 ± 8.6	- 3.4
1000	42.5	40.7	23.7 ± 5.9	7.4
	Adult mortality		Reproduction	
LC_{50} [mg p.m./kg d.wt.s.]	> 1000		-	
LOEC [mg p.m./kg d.wt.s.]	1000		-	
NOEC [mg p.m./kg d.wt.s.]	316		≥ 1000	

p.m. = pure metabolite
- not applicable

After 14 days of exposure, 88.0% corrected mortality according to Abbott (1925) of the adult mites was observed with the reference item group which was within the recommended range of 50.0 - 99.5%.

CONCLUSION

The LC_{50} value was not calculated and estimated as > 1000 mg p.m./kg d.wt.s.
The $NOEC_{Mortality}$ was determined as 316 mg p.m./kg d.wt.s.
The $LOEC_{Mortality}$ was determined as 1000 mg p.m./kg d.wt.s.
The $NOEC_{Reproduction}$ was ≥ 1000 mg p.m./kg d.wt.s.

IIA 8.14.1 Summary of preliminary data: biological activity & dose range finding

Insecticidal activity

Screening data concerning insecticidal activity are not presented.
The relevant information is covered by the guideline studies on representative species, which are presented under the points 8.7 and 8.8 of this section 6.

Further information

Further information on the biological activity of BYI 08330 is given in the respective chapter IIA, point 3.

IIA 8.14.2 Assessment of relevance to potential impact on non-target species

Risk assessments are performed in product-specific Annex III dossiers.

IIA 8.15 Effects on biological methods for sewage treatment

Report:	**KIIA 8.15/01, [redacted]; 2004**
Title:	BYI 08330: Toxicity to bacteria.
	Date: 2004-10-19
Organisation:	Bayer Industry Services GmbH & Co. OHG, Leverkusen, Germany
Report No.:	13129/04 B; M-275364-01-1
Publication:	Unpublished
Dates of experimental work:	September 13, 2004 – September 16, 2004
Guidelines:	Commission Directive 88/302/EEC; Official Journal of the EG L 133 Part C (1988) (mostly identical with OECD 209)
Deviations:	None
GLP:	Yes (certified laboratory)

Executive summary

The study was designed to assess the toxicity of spirotetramat to bacteria. The test guideline Commission Directive 88/302/EEC; Official Journal of the EG L 133 Part C (1988) is in most parts identical with OECD Guideline 209. The method assesses the effect of a test item on microorganisms by measuring the respiration rate under defined conditions in the presence of different concentrations of the test item.

Mixed population of aquatic microorganisms (activated sludge) were exposed to spirotetramat at nominal concentrations of 1000, 1800, 3200, 5600 and 10000 mg a.s./L. The respiration rate of each mixture was measured after an aerated incubation period of 3 hours.
Two controls without test item were included in the test design, one at the start and the other at the end of the test series. Each batch of activated sludge is checked using 3.5-dichlorophenol as reference substance (tested rates: 2.5, 5, 10, 20 and 40 mg/L).
The EC_{50} of BYI 08330 technical for activated sludge is higher than 10000 mg a.s./L.

MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat (BYI 08330) techn.**
Description	**Not specified**
Lot/batch No.	**Batch no.: 08045/0014**
Purity	**97.2%**
Stability of test compound	**Not specified**

2. Vehicle and/or positive control	**3.5-Dichlorophenol was used as reference substance**
3. Test organisms	
Type	**Mixed population of aquatic microorganisms (activated sludge)**
Date of collection	**2004-09-13**
Source	**Aeration tank of a domestic sewage treatment plant (Wupper area water authority, Germany)**
Pre-treatment	**Aeration of the activated sludge; daily feed with synthetic medium**

B Study design and methods

1. In life dates	**September 13, 2004 – September 16, 2004**
2. Test system	
pH	**7.5**
Test temperature	**20 ± 2°C**
Type of application	**Direct weighing**
	The test item has been added to about 130 mL deionised water.
Test concentrations of the activated sludge	**480 mg/L suspended solids**
Stirring period of test item before start of incubation time	**16.5 h (overnight)**
Incubation time	**3 hours with permanent aeration**

3. Observations

The respiration rate of the activated sludge is measured after a contact time of 3 hours. The respiration rate of the same activated sludge in the presence of various concentrations of the test item is also measured. The inhibitory effect of the test item at a particular concentration is expressed as a percentage of the mean of the respiration rates of two controls. An EC_{50} value is calculated from the respiration rates at different test item concentrations.

A physico-chemical oxygen control at 10000 mg/L test item is carried out since some substances may consume oxygen by chemical reactivity. In order to be able to differentiate between physico-chemical oxygen consumption and biological oxygen consumption (respiration), at least the maximum concentration of the test item is additionally tested without activated sludge.

RESULTS AND DISCUSSION

A. Findings

All validity criteria of the test method were met, since the respiratory rates of the 2 controls differed less than 15%, the respiratory rate of the controls was <60 mg O_2/(L × h) and the

EC_{50} of the reference substance 3.5-dichlorophenol was 8.3 mg/L, which was in the range of 5 - 30 mg/L.
The test item and reference substance concentrations are not confirmed by analytical methods; they are based on nominal concentrations.

Test concentration [mg a.s./L]	O_2 start [mg O_2/L]	O_2 end [mg O_2/L]	Exposure time [min.]	Temp. [°C]	pH
1000	5.0	3.5	3	19.5	7.6
1800	5.2	2.9	5	19.5	7.6
3200	5.3	3.5	4	19.6	7.6
5600	3.8	2.5	3	19.5	7.6
10000	5.1	3.2	5	19.6	7.6
Control 1	4.6	3.1	3	19.5	7.7
Control 2	5.1	3.2	4	19.7	7.6
Physico-chemical consumption control	7.7	7.7	9	19.9	7.0

The physico-chemical oxygen consumption has been determined at 10000 mg/L test substance concentration. As no physico-chemical oxygen consumption was observed at that test item concentration this observation also holds true for the lower test item concentrations.

Test concentration [mg a.s./L]	Respiratory rate [O_2/(L × h)]	Phys.-chem. O_2 consumption [O_2/(L × h)]	Respiratory rate - phys.-chem. O_2 consumption [O_2/(L × h)]	Inhibition [%]
1000	30.0	0.0	30.0	0.0
1800	27.6	0.0	27.6	5.8
3200	27.0	0.0	27.0	7.9
5600	26.0	0.0	26.0	11.3
10000	22.8	0.0	22.8	22.2
Control (mean)	29.3			

BYI 08330 technical showed 22.2% respiration inhibition of activated sludge at a test item concentration of 10000 mg/L.

CONCLUSION

The EC_{50} of BYI 08330 technical for activated sludge is higher than 10000 mg a.s./L.

IIA 8.16 Other/special studies

IIA 8.16.1 Other/special studies - laboratory studies

Report:	**KIIA 8.16.1/01, [redacted] 2007**
Title:	Long-Term Mallard Duck Dermal Exposure to BYI 08330. Date: 2007-07-05
Organisation:	[redacted] USA
	Owner: Bayer CropScience
Report No.:	EBFNP033, M-290165-01-1
Publication:	Unpublished
Dates of experimental work:	April 24 2006 - June 19, 2006
Guidelines:	Non-Guideline study
weberDeviations:	-
GLP:	No

Executive summary

This study was designed to collect information on ducks as they are dermally exposed to excreta collected from other ducks that have been exposed to BYI 08330 in their diet. Exposure occurred over eight weeks, and was consistent, with fresh excrement ("treatment excrement") applied every two days. The objective of the study was to determine if dermal exposure to treatment excrement alone is sufficient to elicit a dermal response in the feet of ducks not exposed via the diet. The study was designed with two control and two exposure groups: The dietary control birds only received clean basal feed, the dermal control animals received clean basal feed and the application of dietary control duck excrement to the floor of their cages. The dietary exposure group received treated diet without additional dermal exposure, other than that from their own excreta, on the mesh floor of their cages. The dermal exposure group received clean basal feed and the application of dietary exposure duck excrement to the mesh floor of their cages.

The test substance BYI 08330 technical grade was applied to the dietary exposure group at a nominal dose level of 720 mg a.s./kg feed (mean measured concentration: 760 mg a.s./kg feed). Two sets of ducks were sequentially exposed in this group due to severe reactions necessitating euthanisation of the first set at week 4 of the study. Changes in body weight, detailed foot observations, photographic documentation of foot effects, faecal samples, and collection and preservation of feet from the test system at termination were monitored or collected.

Ducks exposed to BYI 08330 via the diet experienced adverse skin effects on the bottom of their feet observable by 2 weeks after initial exposure. These effects included cracking and drying of the feet, primarily along the digits. Such symptoms were significantly worse after 4 weeks of exposure, when the cracks became severe and significantly impaired mobility. Perceived pain and discomfort in dietarily exposed ducks warranted *in extremis* euthanisation after 4 weeks of exposure. Dietary exposure also significantly affected body weight over the

course of the study, resulting in significant weight loss between each measured interval, and over the course of a 4-week exposure period. The weight loss appeared to result from a combination of avoidance of the treated feed by the test animals and impaired mobility. The onset and severity of the observed effects was consistent in two separate sets of ducks treated sequentially.

Ducks only dermally exposed to excreta of dietarily exposed ducks, consistently over an 8-week period, some birds experienced minor to moderate cracking and drying of the feet, primarily at the mid-point of the study (4 weeks), which was healed by study termination at 8 weeks. However, such cases were isolated and transient, and did not replicate the effects seen in dietarily exposed ducks. In addition, no related effects on general health or behaviour were observed in dermally exposed ducks.

Conclusions: The dermal control and dermal exposure groups all experienced transient occurrences of minor cracking along the foot digits, drying of the skin of the feet, and cracking/sloughing calluses. Such conditions are considered normal. Unique to the dietary exposure group were symptoms of progressively worsening cracking and drying of the feet, and impaired mobility. Dermal exposure alone could not elicit the severe dermal response, behavioural observations and weight changes observed in the high level dietary exposure.

MATERIAL AND METHODS

A Materials

1. Test material	Spirotetramat (BYI 08330) tech.
Description	Not reported
Lot/batch No.	Lot No.: 08045/0014
Purity	98.1%
Stability of test compound	Recertification date: 2006-11-17
2. Vehicle and/or positive control	None
3. Test animals	
Species	Mallard duck (*Anas platyrhynchos*)
Age	35 weeks, 1 day old at experimental start
Source	[redacted] USA
Acclimation period	2 weeks prior to test initiation
Replicates	12 breeding pairs per dose level; one male and one female per pen
Environmental conditions	
Temperature	Room 1: Dermal exposure group 17 – 32 °C, 48 – 87% relative humidity during acclimation, 15 – 33 °C, 57 – 87% relative humidity during the study. Room 2: Dietary and dermal control groups 17 – 28 °C, 41 – 91% relative humidity during

	acclimation, 14 – 29 °C, 46 – 92% relative humidity during the study. Room 3: Dietary and dermal control groups 16 – 28 °C, 64 – 94% relative humidity during acclimation, 15 – 26 °C, 45 – 94% relative humidity during the study.
Photoperiod	7 hours light/17 hours dark

B Study design and methods

1. In life dates April 24, 2006 – June 19, 2006

2. Experimental treatments

All test animals were uniquely identified with numbered leg bands prior to cage assignment. Cages were numbered and male ducks were assigned to cages based upon a computer-generated randomized list of all available cages at test initiation. The first tagged male was placed into the first cage designated in the randomization. The second tagged male was placed into the second designated cage, and so on, until one male was assigned to each cage (cages 1 to 42). Females were matched to male cage mates based on similar body weights. Cages were numbered sequentially, beginning in room 1. Because groups were assigned by room, animals were assigned to groups as they were assigned to a cage.

Prior to treatment, body weights among groups were compared to ensure normality, homogeneity, and no significant differences among groups.

The ducks included in the second dietary exposure set were treated immediately following assignment to test cages, and had been acclimated in similar caging.

Birds were fed with Purina® Flight Conditioner®. Feed was mixed with the test substance BYI 08330 technical grade to achieve a nominal dose level of 720 mg a.s./kg feed. The treated diet mix was stored under frozen conditions. Untreated basal feed was given to the controls. Analyses were conducted to confirm appropriate diet concentration of each diet mix prepared. Dietary analysis confirmed recoveries of 95 to 116% of nominal, resulting in an average measured dietary concentration of 760 mg a.i./kg feed. Feed and water was provided to the test organisms *ad libitum*. All birds proceeding into the experimental period were in apparent good health.

During the experimental period, plastic catch trays with shallow sides were placed under each individual cage to catch all excrement. Every day, all cages (front, sides, upper surface of floor, and underside of floor) were scrubbed with brushes dipped in clean water, and the catch trays emptied, scrubbed, and rinsed. The excrement from the dietary control and dietary exposure cages was collected after the cages were scrubbed, prior to cleaning of the catch trays. The cages of the dermal control and dermal exposure cages were scrubbed prior to application of fresh excrement from the appropriate dietary cage. The catch trays from the dermal cages were scrubbed and rinsed after application of excrement to the cage floor.

Separate equipment (i.e.: carts, cleaning equipment, protective foot coverings, etc.) were used in each room.

Excrement collected from each dietary control and dietary exposure cage was collected in separate small buckets. The excrement was then transferred to the cage floor of the corresponding dermal control or dermal exposure cage.
Ducks in the dermal groups (control and exposure) received initial exposure to the corresponding dietary group excrement two days after dietary exposure initiation. The birds in the dietary exposure group were fed treated feed for 4 weeks. Because severe foot ailment and body weight loss were observed in the first dietary exposure group, the initial set of dietary exposure animals was euthanized at four weeks and was replaced with untreated ducks from the original colony. The replacement ducks were also fed treated feed for four weeks, after which time the study was terminated.

3. Observations

Behavioral and detailed foot observations

Each animal was generally observed daily during room maintenance procedures. During these observation periods unusual behaviours were noted. On the day of treatment and at the end of each two-week interval thereafter, all birds were captured and their feet closely examined. During this examination, notations were made in greater detail regarding unique conditions of the birds' feet, and digital photos were taken of each individual animal's feet.

Excrement sampling

Composite excrement samples were collected from the dietary exposure group to confirm that the dermal exposure ducks were receiving exposure similar to that experienced by the dietary exposure ducks as they walked about their cage. Composite excrement samples were collected from dietary control group to verify lack of exposure in the control groups, and were archived for analysis as necessary. The resulting values from analyses of the excrement samples are intended to provide a general idea of the degree of exposure, not for precise quantification of the test material concentration in the excrement.
One composite excrement sample was collected from the dietary control and dietary exposure group every two weeks following treatment initiation. Individual samples were collected from each cage in each group, and combined, by group, to create the composite sample. The samples were collected prior to excrement collection for transfer to the dermal exposure group cages. Care was taken to avoid feed and excess water in the excrement samples and the excrement collected for transfer to the dermal exposure or control cages. Samples were stored frozen immediately after collection, and were shipped on ice to the analytical laboratory.

Body weight determination

Body weights were measured for each animal on the day of cage assignment, at treatment initiation, and at the end of each two-week interval thereafter. Prior to test initiation, statistical comparisons were made among groups to ensure that all groups exhibited similar mean weights and that body weights had similar variances among groups.
Body weight data taken prior to the start of treated feed was analyzed for homogeneity of variance among cages using Levene's Test. Body weights were compared using one-way analysis of variance to test for mean differences among groups.
At the end of the study, calculated proportional changes in body weight were compared

among groups. Because there were two subsequent sets of ducks tested in the dietary exposure group, proportional body weight change from day 0 to week 2, and from day 0 to week 4 were compared among groups including the first dietary exposure set, and proportional body weight change from week 4 to week 6, and from week 4 to week 8 were compared among groups including the second dietary exposure set. Proportional body weight change values were not arc-sin transformed prior to analysis due to values greater than 1 or less than 0. Weight change data was first tested for normality using Shapiro-Wilk's test and homogeneity of variance using Levene's Test. Data were analyzed for mean differences among groups using analysis of variance (ANOVA) followed by either Dunnett's or Bonferroni's t-Test for mean pair-wise comparisons. All analyses were conducted in TOXSTAT® version 3.5.

RESULTS AND DISCUSSION

A. Findings

Results of the foot observations and change in body weight during the study are summarised in the following tables.

Table 1 Foot observations of Mallard duck (*Anas platyrhynchos*) after dermal exposure with BYI 08330

		Week			
Group	**Observation**	**2**	**4**	**6**	**8**
Dietary control (n=24)	Cases of moderate cracking	0	0	0	0
	Cases of severe cracking	0	0	0	0
	Cases of dry skin	0	0	0	0
	Cases of cracked calluses	0	0	0	0
	Cases of inflamed webbing	0	0	0	0
	Number of affected birds[a]	**0**	**0**	**0**	**0**
Dermal control (n=24)	Cases of Moderate cracking	0	0	0	0
	Cases of severe cracking	0	0	0	0
	Cases of dry skin	0	0	0	0
	Cases of cracked calluses	0	0	0	0
	Cases of inflamed webbing	0	0	0	0
	Number of affected birds	**0**	**0**	**0**	**0**
Dietary exposure 1 (n=24)	Cases of moderate cracking	0	11	-	-
	Cases of severe cracking	0	13	-	-
	Cases of dry skin	0	17	-	-
	Cases of cracked calluses	4	0	-	-
	Cases of inflamed webbing	0	7	-	-
	Number of affected birds	**4**	**24**	**NA***	**NA***
Dietary exposure 2 (n=24)	Cases of moderate cracking	-	-	4	5
	Cases of severe cracking	-	-	2	14
	Cases of dry skin	-	-	0	6
	Cases of cracked calluses	-	-	0	0
	Cases of inflamed webbing	-	-	0	0
	Number of affected birds	**NA****	**NA****	**6**	**21**
Dermal exposure (n=24)	Cases of moderate cracking	0	4	1	0
	Cases of severe cracking	0	0	0	0
	Cases of dry skin	0	1	1	1
	Cases of cracked calluses	2	0	1	0
	Cases of inflamed webbing	0	1	0	0
	Number of affected birds	**2**	**6**	**2**	**1**

* Not applicable, birds were euthanised 4 weeks after exposure

** Not applicable, birds replaced the euthanised animals 4 weeks after initiation of the study

[a] Total number of observations may exceed total number of affected birds due to certain individuals exhibiting more than one abnormal symptom during a given observation interval.

Table 2 Body weight of Mallard duck (*Anas platyrhynchos*) after dermal exposure with BYI 08330

	Dermal control		Dietary control		Dermal exposure		Dietary exposure	
Average body weight	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Day -7 [g]	1126.3	1080.1	1175.7	1156.4	1119.1	1071.3	1136.1	1098.4
Time 0 [g]	1065.9	1020.5	1107.0	1096.9	1053.8	1032.4	1070.7	1020.6
Week 2 [g]	1134.5	1006.4	1163.3	1091.2	1135.2	998.0	1016.4	952.5
Proportional change Day 0 - Week 2	0.06	-0.01	0.05	0.00	0.08	-0.03	-0.05 [a]	-0.07 [a]
Week 4 [g]	1173.6	1084.1	1228.0	1147.0	1185.6	1038.2	1035.6	998.5
Proportional change Day 0 - Week 4	0.10	0.07	0.11	0.05	0.12	0.01	-0.08 [a]	-0.11 [a]
Week 6 [g]	1167.5	996.3	1232.5	1076.4	1161.2	1008.0	933.7	901.7
Proportional change Week 4 - Week 6	-0.01	-0.08	0.00	-0.06	-0.02	-0.04	-0.14 [a]	-0.16 [b]
Week 8 [g]	1191.7	984.2	1237.0	1092.6	1160.3	1024.1	876.0	888.1
Proportional change Week 4 - Week 8	0.01	-0.09	0.01	-0.04	-0.02	-0.02	-0.19 [a]	-0.18 [b]

[a] Significantly reduced compared to the control, based on Dunnett's Test

[b] Significantly reduced compared to the control, based on Bonferroni's Test

B. Observations

Behavioural observations

No abnormal behavioural observations were recorded for birds in either the dietary control or dermal control group. One female in the dermal exposure group was observed to be emaciated, lethargic, weak, lacrimating, and to have diminished appetite and green, runny excrement. This was first observed during week 5. Separating the female from her cage mate did not appear to aid in her ability to access food, and as her weakness was pronounced, she was subsequently euthanised. However, the condition of her feet were normal, and her symptoms generally were not similar to those observed in the dietary exposure group, therefore her condition is not considered to have been due to exposure to the treated excrement. No other abnormal behaviours were noted among birds in the dermal exposure group.

Ducks in both dietary exposure sets (week 0 to 4 and week 4 to 8) displayed abnormal behaviour and adverse reactions in their feet. Behavioural effects developed in the dietary exposure group are as follows: Limping/favouring one foot over another, goose-stepping (exaggerated high steps), unsteady when walking/indication of sensitive or tender feet, decreased ambulation, not walking, using wings to aid locomotion, piloerection, weak eyes, lacrimation, or deposits around eyes, cracking on feet visible without handling bird (cracks wrap around edges of feet), stumbles often when walking and emaciation. Though each set of

dietary exposure ducks were euthanized after four weeks of exposure, no fatalities occurred in this group during the study.

Foot observations

Dietary control ducks did not experience any cracking or drying during the 8-week course of this study (see Table 1). However, two notations were made of minor cuts along the foot digits of ducks in the dermal control group. Because such an occurrence was noted in the control group, it was considered normal, further, it is not unusual for ducks to experience temporary cracking and healing of the skin of their feet from time to time. In all cases, raised, dry calluses are considered normal, as they are often observed by study personnel in ducks under a variety of conditions.

In the dermal exposure group, four occurrences of minor cracking along the digits were noted four weeks apart, and three occurrences of moderate cracking along the digits were noted during the middle two weeks of the study. These notations did not always correspond with those of dry webbing. The noted effects in this group seems to have been transient as the moderate notations either improved to minor or were healed by week 8. During week 4, 10 cases of inflamed webbing were noted. This effect seems to have been transient, as it was not apparent by week 6.

Upon examination of the feet of the birds in the dietary exposure group, the likely cause of their abnormal behaviours could be observed. The condition of the ducks' feet in the dietary exposure group commonly included moderate to extreme dermal cracking along the digits, drying of the skin along the digits as well as the webbing between the digits, and impaired mobility and perceived discomfort due to the sores on the feet. Some of the cracking became so deep that the cracks extended around the digits, becoming visible from the side without handling the bird. This severe cracking was distinctly different from transient minor cracking observed in the controls and dermal exposure groups. This group also experienced a transient effect of inflamed webbing during week 4, with 24 birds affected (twice as many as in the dermal exposure group). The inflammation was gone by week 6, as in the dermal exposure group. Some birds became reluctant to feed and became weak and emaciated.

Excrement sample analysis

Generally, analyses showed the presence of both the parent BYI 08330 (0.11 to 0.60 ppm), and BYI 08330-enol (61.6 to 94.5 ppm). The analytical data confirmed that the exposure via dermal contact with the excreta was similar between the dietary exposure group and the dermal exposure group. The analysis also confirmed that the levels of BYI 08330 and BYI 08330-enol in the excreta of in the first group of birds given treated diet was similar to that in the excreta of the second group of birds on treated diet. The control samples analyzed revealed concentrations below the detectable limit (<0.01 ppm) for both BYI 08330 and BYI 08330-enol.

Body weight

The results of the body weight measurements are summarised in Table 2.

At test initiation, body weights among all groups were normally distributed and had homogeneous variances. No significant mean differences in pre-treatment body weight were observed between the control and exposure groups. Both genders within the first set of dietary

exposure ducks gained significantly less weight (generally lost weight) during the day 0 – week 2 measurement interval (female f = 2.5963, critical f = 2.8165, p = 0.0.0643; male f = 16.2810, critical f = 2.8165, p = 0.0000) and overall from day 0 – week 4 (female f = 10.0806, critical f = 2.8165, p = 0.0000; male f = 25.3069, critical f = 2.8165, p = 0.0000). The second set of dietary exposure ducks experienced similar weight loss, resulting in significantly different body weight changes during the interval of week 4 to 6 (female f = 99.5205, critical f = 2.8216, p = 0.0001; male f = 51.2227, critical f = 2.8165, p = 0.0000) and week 4 to 8 (female f = 8.9917, critical f = 2.8216, p = 0.0001; male f = 55.1858, critical f = 2.8265, p = 0.0000). These patterns of weight loss correlate to general observations of the ducks' reluctance to feed, as observed during the study.

CONCLUSION

The dermal control and dermal exposure groups all experienced transient occurrences of minor cracking along the foot digits, drying of the skin of the feet, and cracking/sloughing calluses. Such conditions are considered normal. Unique to the dietary exposure group were symptoms of progressively worsening cracking and drying of the feet, and impaired mobility. Two separate sets of ducks exposed to the test material at 720 mg a.s./kg feed exhibited these symptoms within 2 weeks of initial dietary exposure to the test material, and symptoms progressed to warrant *in extremis* euthanisation of both sets after 4 weeks of continual dietary exposure. Ducks dermally exposed to the excrement of dietarily exposed ducks experienced fewer cases of minor to moderate cracking and drying of the feet during 8 weeks of repeated exposure to the excrement, and symptoms were transient, less severe, and did not lead to behavioural symptoms or other signs indicating that such conditions negatively affected their health. Dermal exposure alone could not elicit the severe dermal response, behavioural observations and weight changes observed in the high level dietary exposure.

IIA 8.16.2 Other special studies - field studies

Report:	**KIIA 8.16.2/01, [redacted]; 2008**
Title:	Residues of Spirotetramat in Arthropods After Spray Application in Citrus – Magnitude and Time Course of Residue Decline.
	Date: 2008-01-09
Organisation:	RIFCON GmbH, Heidelberg, Germany
	Owner: Bayer CropScience
Report No.:	RA07031, M-296043-01-1
Publication:	Unpublished
Dates of experimental work:	May 27, 2007 – June 18, 2007
Guidelines:	Non-Guideline Study
Deviations:	-
GLP:	yes

Executive summary

The aim of the field study was to determine residue levels of Spirotetramat (BYI 08330) and its metabolites BYI 08330-enol and BYI 08330-ketohydroxy on foliage and ground dwelling arthropods in citrus orchards, after spray application of Spirotetramat OD 150 B G, in order to assess the risks to insectivorous and omnivorous birds and mammals, which may feed on these arthropods.

The field study was conducted in citrus orchards in the region of Valencia in Eastern Spain, a typical area of citrus cultivation in Southern Europe. The test site was situated near Gandia and consisted of two orchards (1.2 and 1.3 ha) with two plots each (a total of four replicates; approx. 0.6 ha each).

The test item was applied separately on each plot once at an application rate of 99.6 and 121.3 g a.s. per ha and meter canopy height (which is equivalent to nominal 270 g a.s./ha in all cases) with a spray volume of 3000 L/ha.

Ground dwelling arthropods were sampled using pitfall traps. Foliage dwelling arthropods were collected by inventory spray sampling. Trees were sprayed with the knockdown insecticide Aquapy containing 30 g natural Pyrethrum L at an concentration of 1.5 g a.s./L.

Sampling was carried out one day before application of the test substance, i.e. day after treatment (DAT) -1 and on DAT 0 (before application, ground dwellers). After the application, samples were taken on DAT 0 (foliage dwellers), +1, +2, +3, +4, +5, +7, +9, +11, +14 and +21. After swift identification and quantification of the main taxonomic groups the samples were stored deep frozen until residue analysis.

Initial and maximum measured values of the mean residues (n = 4) were expressed for Spirotetramat (parent), Spirotetramat+BYI 08330-enol (parent+enol) and Spirotetramat+BYI 08330-enol+BYI08330-ketohydroxy (total measured residue). Mean residue values of the parent, parent+enol and total measured residue were used to calculate the time weighted averages (TWAs) of daily intervals between DAT 1-21 (ground dwellers) and DAT 0-21 (foliage dwellers), expressed as percentage of the maximum measured residue value.

The residue decline (DT_{50}) of the parent, parent+enol and total measured residue in foliage dwelling arthropods (DAT 0-21) was determined to assess the potential exposure of foliage dwelling arthropods to the active substance. The data used are based on the arithmetic mean of the residue values of four replicates. The residue decline was considered to follow a first-order kinetic.

Results are summarised in Table 1.

Table 1 Residues of BYI 08330 and its metabolites BYI08330-enol and BYI08330-ketohydroxy in ground and foliage dwelling arthropods after spray application of 270 g a.s./ha in citrus

Mean residue concentrations [mg/kg f.w.] in ground dwelling arthropods			
Ground dwellers	Parent	Parent+enol	Total measured residue
Initial measured value (DAT +1)	0.21	0.52	0.54
Maximum measured value (MMV)	0.32 (DAT +2)	0.60 (DAT +2)	0.62 (DAT +2)
$TWA_{(DAT\ 1-21)}$ long term risk	0.15 (46% of MMV)	0.35 (59% of MMV)	0.39 (63% of MMV)
Mean residue concentrations [mg/kg f.w.] in foliage dwelling arthropods			
Foliage dwellers	Parent	Parent+enol	Total measured residue
Initial measured value (DAT +0)	4.0	5.2	5.3
Maximum measured value (MMV)	4.0 (DAT +0)	6.0 (DAT +1)	6.2 (DAT +1)
$TWA_{(DAT\ 0-21)}$ long term risk	1.07 (27% of MMV)	1.90 (32% of MMV)	2.03 (33% of MMV)
DT_{50} [d]	2.8	4.7	5.1

f.w.: fresh weight

Conclusions: The study provides realistic data on magnitude of peak residue levels and the corresponding time course of residue decline of Spirotetramat and its metabolites BYI 08330-enol and BYI 08330-ketohydroxy in ground and foliage dwelling arthropods. Maximum measured residues in ground dwellers were 0.32, 0.60 and 0.62 mg/kg fresh weight (f.w.) for parent, parent+enol and total measured residues, respectively. In foliage dwellers maximum measured residues were ten times higher than in ground dwellers, with 4.0, 6.0 and 6.2 mg/kg f.w. for parent, parent+enol and total measured residues, respectively. The decline of the residue levels of parent, parent+enol and total measured residue in foliage dwellers followed a first-order kinetic. The DT_{50} for residue dissipation was 2.8, 4.7 and 5.1 days for parent, parent+enol and total measured residue respectively. These data provide a reliable basis for use in higher tier risk assessments of insectivorous and omnivorous birds and mammals.

MATERIAL AND METHODS

A Materials

1. Test material — Spirotetramat OD 150B G

Description	150 g/L Spirotetramat (BYI 08330), density: 0.986 g/mL, light beige suspension
Lot/batch No.	Batch No. 2007-0002474
Purity	15.5% w/w 152.8 g a.s./L
Stability of test compound	Expiry date: 2008-04-10
2. Vehicle and/or positive control	Vehicle: Basal bird feed Positive control: none
3. Test animals	Natural arthropod populations of citrus orchards, which potentially serve as prey for insectivorous and omnivorous birds and mammals
Species	-
Age	
Source	Citrus orchards near Gandia, Spain
Acclimation period	-
Replicates	4
Environmental conditions	4 Rainfall events on DAT +8, +13, +15 and +20
Temperature	Minimum daily temperature ranged between 14 and 24 °C (mean 18 °C), maximum daily temperature fluctuated between 23 and 33 °C (mean 27 °C)
Photoperiod	Natural photoperiod

B Study design and methods

1. In life dates: May 23, 2007 - June 18, 2007

2. Experimental treatments

The field study was conducted in citrus orchards in the vicinity of Xeraco near Gandia, Eastern Spain. Two commercially used orchards with a size of 1.2 and 1.3 ha were selected to establish four plots. Each plot consisted of 10 rows of trees (between 514 up to 709 trees per plot) with a length between 101 m up to 191 m. The test item was applied on plot 1+2 and plot 3+4 with an application rate of 99.6 and 121.3 g a.s./(ha*m canopy height) (which is equivalent to nominal 270 g a.s./ha in all cases), respectively, and with a spray volume of 3000 L/ha. The canopy height of the trees in each replicate was 2.5 - 3.0 m, mean 2.8 m (plot 1+2) and 2.0 - 2.5 m, mean 2.3 m (plot 3+4) respectively.

In order to obtain arthropods from the study plots, samples were collected separately from two different strata within the citrus orchards: ground and foliage. For this purpose, ground dwelling arthropods were sampled by using pitfall traps and the foliage was sprayed with a 'knockdown' insecticide such as Aquapy (active substance content: 30 g natural Pyrethrum/L) to obtain foliage dwelling arthropods (inventory spraying). The number of pitfall traps set up on plot 1 and 2 was 86 each, and for plot 3 and 4 it was 90. The traps were placed within the rows of the orchards, in the middle of two trees, in an adequate and evenly distributed set-up at a distance of approximately 10 m from trap to

trap, starting approx. 5 m from the ends of the row. Edge rows were left out. To avoid Spirotetramat contamination of the traps the plastic pipes were closed with appropriate covers (diameter of approx. 11 cm during the applications). At the sampling dates, all traps of one plot were emptied into one 50 mL polypropylene sampling bottle. Thus there is one sample per plot and per sampling event. The procedure of emptying the pitfall traps was conducted 24 h after activating the traps to catch diurnal and nocturnal arthropods.
For the foliage dwellers sampling, the 'knockdown' insecticide was applied at an concentration of 1.5 g a.s./L with a motor driven knapsack sprayer until shortly before the point of run-off. Edge rows and trees at the beginning or end of a row were left out.
Approx. 10 - 30 min prior to treatment, thin cotton sheets were placed on each side of the row, such that arthropods falling from the leaves drop onto them.
In total four to eight trees were sampled per sampling date and replicate. The sampled trees were marked with red and white striped tape and recorded in the raw data to avoid repetition of sampling in the same area. Approximately two hours after each application of Aquapy all arthropods that had fallen onto the sheets were pooled into a 50 mL polypropylene sample bottle.
Any arthropods caught alive were killed immediately after sampling with ether, as ether does not interfere with the residue detection method.
Immediately after sampling the composition of every single sample was determined in the field, in terms of main taxonomic groups (e.g. Coleoptera, Arachnida, Chilopoda, Isopoda, Dermaptera, Diptera and Hymenoptera), subdivided into adults and larval stages. Speed of sample handling was optimized to reduce desiccation of the sample material during hot weather. The number of individuals of each taxonomic group was estimated (frequency classes: 1, 2-5, 6-10, 11-25, 26-50, 51-100, 101-200, 201-300 individuals per sample). Also to ensure a short handling time, the pitfall trap samples were sieved (in order to remove sand in the samples) before sorting the arthropods from DAT +1 until the end of the study.
All samples were frozen on dry ice immediately after taxonomic identification in the field at <-40 °C.
Initial measured values (IMV) and maximum measured values (MMV) of the mean residues of four replicates were expressed for parent, parent+enol and total measured residue.
The time weighted average (TWA) is a measure which describes a concentration (e.g. residue values) averaged over a given time span (e.g over 21 days). Graphically, TWA is represented by the area under the curve (AUC) of the time series plot of a function.
TWAs of residue values were calculated by interpolating values for days for which no experimental data were available (linear interpolation) and by calculating the mean over a given time period. TWAs of residue values of the parent, parent+enol and total measured residue were calculated for daily intervals (moving window) between DAT 1-21 and DAT 0-21, for ground dwelling and leaf dwelling arthropods, respectively. The TWAs were expressed as percentage of MMV.
TWA calculations and the calculation of the IMV were carried out with MS Office Excel 2003.

The residue decline (DT_{50}) of parent, parent+enol and total measured residue in leaf dwelling arthropods (DAT 0-21) was estimated to assess the potential exposure of leaf dwelling arthropods to the active substance. The data used are based on the arithmetic mean of the residue values of four replicates. It was considered that the residue decline for leaf dwelling arthropods followed a first-order kinetic.
The DT_{50} was calculated using KinGUI Version 0.6.
3. Observations
Inventory spraying was conducted once before the first application of the test substance (collection of control samples for residue analysis, quantification of arthropod abundance and to adjust the number of trees considered necessary per sampling event), i.e. on DAT -1. Further samples were taken on DAT 0 (after application), +1, +2, +3, +4, +5, +7, +9, +11, +14 and +21.
Pitfall trapping was conducted 12 times per plot, starting one day (24 h) before the first application of the test substance, i.e. day after treatment (DAT) -1. Further samples were taken on DAT 0 (before application), +1, +2, +3, +4, +5, +7, +9, +11, +14 and +21.

RESULTS AND DISCUSSION

A. Findings

Analytical findings

The individual recovery values for BYI 08330 ranged from 87 to 97%, with an overall recovery of 92% and a relative standard deviation (RSD) of 3.2% (n = 12). The individual recovery values for BYI 08330-enol ranged from 93 to 104%, with an overall recovery of 99% and RSD of 3.5% (n = 12). The individual recovery values for BYI 08330-ketohydroxy ranged from 72 to 91%, with an overall recovery of 79% and RSD of 8.6% (n = 10). All results of the method validation were in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully.
The Limit of Quantification (LOQ), defined as the lowest validated fortification level, was 0.01 mg/kg for BYI 08330 and its metabolites BYI 08330-enol and BYI 08330-ketohydroxy in/on arthropods.

Biological findings

The number of individuals per plot and the taxonomic groups of arthropods living in citrus orchards are presented in Table 2 to 9.

Table 2 Number of individuals of ground dwelling arthropods (Plot 1)

Plot 1	Number of individuals per sampling day (ground dwellers)												
Taxonomic group	-1	-0	+1	+2	+3	+4	+5	+7	+9	+11	+14	+21
Arachnida	11-25	11-25	11-25	11-25	26-50	11-25	26-50	26-50	11-25	11-25	11-25	26-50
Isopoda	-	-	1	2-5	2-5	2-5	1	2-5	2-5	1	2-5	2-5
Myriapoda	6-10	11-25	11-25	6-10	6-10	2-5	11-25	26-50	26-50	11-25	11-25	11-25
Ephemeroptera	-	-	-	-	-	-	-	-	-	-	-	-
Odonata	-	-	-	-	1	-	-	1	-	-	-	-
Saltatoria	-	-	-	-	1	-	-	-	-	-	-	-
Dermaptera	1	-	-	-	2-5	-	2-5	2-5	2-5	2-5	2-5	2-5
Heteroptera	-	-	-	-	1	-	1	-	-	-	-	-
Aphidina	-	-	-	-	-	-	-	-	-	-	-	-
Coleoptera	6-10	2-5	2-5	6-10	11-25	6-10	6-10	6-10	11-25	2-5	6-10	2-5
Coleoptera larvae	-	-	-	-	-	-	-	-	-	-	-	-
Neuroptera	-	-	-	1	1	-	-	-	-	-	-	-
Neuroptera larvae	-	-	-	-	-	-	-	-	-	-	-	-
Hymenoptera*	-	-	1	2-5	1	-	1	2-5	2-5	-	1	-
Formicidae	-	-	-	1	2-5	-	1	1	2-5	2-5	6-10	-
Diptera	1	-	1	1	-	-	2-5	1	-	-	1	-
Lepidoptera	1	-	-	-	-	2-5	-	-	1	1	-	-
Lepidoptera larvae	2-5	11-25	6-10	2-5	2-5	6-10	2-5	6-10	2-5	2-5	-	2-5

*Hymenoptera without Formicidae

Table 3 Number of individuals of ground dwelling arthropods (Plot 2)

Number of individuals per sampling day (ground dwellers)													
-1	**-0**	**+2**	**+3**	**+4**	**+5**	**+7**	**+9**	**+11**	**+14**	**+21**
11-25	11-25	6-10	11-25	26-50	26-50	26-50	26-50	11-25	11-25	26-50
26-50	26-50	51-100	101-200	101-200	101-200	101-200	101-200	101-200	101-200	201-300
6-10	11-25	6-10	2-5	6-10	6-10	11-25	11-25	11-25	11-25	6-10
-	-	-	-	-	1	-	-	-	-	-
-	-	1	-	-	-	-	-	-	-	-
-	-	-	-	-	2-5	-	-	-	-	-
-	-	2-5	1	-	-	-	2-5	2-5	6-10	11-25
1	-	-	-	1	-	6-10	2-5	-	-	-
2-5	2-5	11-25	6-10	6-10	6-10	6-10	2-5	6-10	11-25	11-25
-	-	-	-	-	1	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	1	-	-	1	2-5	1	1	1
11-	-	-	1	1	1	1	2-5	1	6-10	-
2-5	1	1	-	1	1	1	2-5	1	-	1
-	-	1	-	-	1	-	-	-	-	-
2-5	2-5	6-10	6-10	6-10	2-5	2-5	2-5	6-10	2-5	1

Plot 2	
Taxonomic group	
Arachnida	
Isopoda	
Myriapoda	
Ephemeroptera	
Odonata	
Saltatoria	
Dermaptera	
Heteroptera	
Aphidina	
Coleoptera	
Coleoptera larvae	
Neuroptera	
Neuroptera	
Hymenoptera*	
Formicidae	
Diptera	
Lepidoptera	
Lepidoptera	

*Hymenoptera without Formicidae

Table 4 Number of individuals of ground dwelling arthropods (Plot 3)

Plot 3	Number of individuals per sampling day (ground dwellers)												
Taxonomic group	-1	-0	+1	+2	+3	+4	+5	+7	+9	+11	+14	+21
Arachnida	26-50	11-25	6-10	26-50	26-50	26-50	26-50	26-50	26-50	26-50	11-25	26-50
Isopodas	2-5	-	2-5	1	2-5	2-5	-	1	2-5	-	2-5	2-5
Myriapoda	6-10	2-5	2-5	6-10	2-5	2-5	11-25	2-5	26-50	6-10	11-25	6-10
Ephemeroptera	-	-	-	-	-	-	1	-	-	-	-	-
Odonata	-	-	-	-	-	-	-	-	-	-	-	-
Saltatoria	-	-	1	-	-	-	-	-	-	-	-	-
Dermaptera	2-5	2-5	2-5	2-5	2-5	2-5	6-10	6-10	6-10	6-10	6-10	11-25
Heteroptera	-	-	-	-	-	-	-	-	-	-	-	-
Aphidina	2-5	-	-	2-5	-	-	-	-	-	-	-	-
Coleoptera	11-25	2-5	2-5	6-10	11-25	2-5	11-25	6-10	6-10	11-25	11-25	6-10
Coleoptera larvae	-	-	-	-	-	-	-	-	-	-	1	-
Neuroptera	-	-	-	-	-	-	-	-	-	-	-	-
Neuroptera larvae	-	-	-	-	1	-	-	-	-	-	-	-
Hymenoptera*	1	-	-	1	1	2-5	2-5	2-5	2-5	-	1	2-5
Formicidae	11-25	2-5	-	1	2-5	2-5	2-5	-	2-5	1	6-10	2-5
Diptera	2-5	-	-	-	-	-	-	-	-	-	1	-
Lepidoptera	-	-	-	-	-	-	1	-	-	-	-	-
Lepidoptera larvae	2-5	2-5	6-10	2-5	2-5	6-10	2-5	6-10	2-5	6-10	1	-

*Hymenoptera without Formicidae

Table 5 Number of individuals of ground dwelling arthropods (Plot 4)

Plot 4	Number of individuals per sampling day (ground dwellers)												
Taxonomic group	-1	-0	+1	+2	+3	+4	+5	+7	+9	+11	+14	+21
Arachnida	6-10	11-25	11-25	26-50	26-50	26-50	26-50	26-50	26-50	26-50	11-25	26-50
Isopodas	6-10	6-10	11-25	11-25	11-25	11-25	6-10	11-25	6-10	6-10	6-10	11-25
Myriapoda	11-25	11-25	6-10	6-10	11-25	2-5	11-25	11-25	11-25	6-10	11-25	2-5
Ephemeroptera	-	-	-	-	-	-	-	-	-	-	-	-
Odonata	-	-	-	-	-	-	-	-	-	-	-	-
Saltatoria	-	1	-	-	-	-	1	1	-	-	-	-
Dermaptera	6-10	6-10	6-10	2-5	6-11	2-5	6-10	6-10	11-25	2-5	11-25	26-50
Heteroptera	-	-	-	-	-	-	-	-	-	-	-	-
Aphidina	-	-	-	-	-	-	-	-	-	-	-	-
Coleoptera	11-25	11-25	6-10	11-25	6-11	11-25	6-26	6-10	11-25	11-25	26-50	11-25
Coleoptera larvae	-	-	-	-	-	-	1	-	-	-	-	-
Neuroptera	-	-	-	-	-	-	-	-	-	-	-	-
Neuroptera larvae	-	-	-	-	-	-	-	-	-	-	-	-
Hymenoptera*	-	-	-	2-5	1	2-5	1	1	1	-	-	-
Formicidae	11-25	1	2-5	2-5	2-5	1	1	1	1	1	2-5	1
Diptera	-	-	-	-	-	-	-	-	-	-	-	-
Lepidoptera	-	-	-	-	-	-	-	-	-	-	-	-
Lepidoptera larvae	2-5	6-10	1	6-10	6-11	2-5	6-11	2-5	2-5	2-5	1	2-5

*Hymenoptera without Formicidae

Table 6 Number of individuals of foliage dwelling arthropods (Plot 1)

Plot 1	Number of individuals per sampling day (foliage dwellers)											
Taxonomic group	-1	+0	+2	+3	+4	+5	+7	+9	+11	+14	+21
Arachnida	6-10	2-5	6-10	11-25	6-10	6-10	6-10	11-25	6-10	11-25	6-10
Isopoda	-	-	-	-	-	-	-	-	-	-	-
Myriapoda	-	-	-	-	-	-	-	-	-	-	-
Ephemeroptera	26-50	11-25	26-50	101-200	26-50	51-100	201-300	201-300	201-300	301-400	301-400
Odonata	-	-	6-10	2-5	2-5	2-5	2-5	11-25	6-10	11-25	-
Saltatoria	6-10	6-10	11-25	6-10	2-5	2-5	6-10	11-25	11-25	11-25	2-5
Dermaptera	-	-	-	-	-	-	-	-	-	-	-
Heteroptera	2-5	1	2-5	2-5	2-5	11-25	6-10	6-10	2-5	6-10	6-10
Aphidina	11-25	11-25	11-25	11-25	11-25	11-25	11-25	11-25	-	-	-
Coleoptera	11-25	26-50	26-50	11-25	11-25	11-25	11-25	26-50	26-50	26-50	26-50
Coleoptera larvae	-	-	-	-	-	-	-	-	-	-	-
Neuroptera	6-10	6-10	11-25	11-25	6-10	2-5	2-5	6-10	11-25	11-25	6-10
Neuroptera larvae	-	26-50	6-10	6-10	6-10	2-5	6-10	2-5	6-10	6-10	2-5
Hymenoptera*	6-10	11-25	6-10	2-5	2-5	11-25	6-10	11-25	6-10	26-50	11-25
Formicidae	1	6-10	-	-	1-5	51-100	51-100	6-10	1-5	2-5	2-5
Diptera	11-25	51-100	6-10	26-50	26-50	51-100	51-100	101-200	51-100	51-100	101-200
Lepidoptera	-	-	-	-	1	2-5	6-10	11-25	11-25	6-10	11-25
Lepidoptera	2-5	2-5	1	-	-	-	-	-	-	-	-

*Hymenoptera without Formicidae

Table 7 Number of individuals of foliage dwelling arthropods (Plot 2)

Number of individuals per sampling day (foliage dwellers)													
-4	+0	+2	+3	+4	+5	+7	+9	+11	+14	+21
2-5	2-5	11-25	6-10	6-10	11-25	11-25	11-25	6-10	26-50	6-10
-	-	-	-	-	-	-	2-5	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	11-25	26-50	101-200	51-100	201-300	101-200	101-200	101-200	101-200	51-100
1	6-10	2-5	6-10	6-10	6-10	6-10	11-25	26-50	101-200	11-25
-	1	11-25	6-10	1-25	6-10	2-5	11-25	11-25	26-50	26-50
1	1	1	2-5	-	-	-	-	1	-	-
1	1	1	-	-	-	-	-	1	6-10	6-10
11-25	26-50	11-25	6-10	-	26-50	6-10	11-25	-	-	-
11-25	26-50	11-25	11-25	11-25	26-50	11-25	26-50	26-50	26-50	11-25
-	-	-	-	-	-	-	-	-	-	-
6-10	2-5	6-10	6-10	11-25	11-25	6-10	11-25	6-10	11-25	2-5
6-10	11-25	6-10	6-10	11-25	11-25	6-10	11-25	11-25	11-25	26-50
-	2-5	6-10	11-25	2-5	11-25	6-10	11-25	-	6-10	-
-	-	-	-	1	26-50	-	-	-	-	-
11-25	6-10	51-100	26-50	26-50	51-100	51-100	51-100	51-100	51-100	51-100
-	-	1	2-5	2-5	2-5	6-10	11-25	11-25	11-25	11-25
1	-	-	-	-	-	-	-	-	-	-

Plot 2	
Taxonomic group	
Arachnida	
Isopoda	
Myriapoda	
Ephemeroptera	
Odonata	
Saltatoria	
Dermaptera	
Heteroptera	
Aphidina	
Coleoptera	
Coleoptera larvae	
Neuroptera	
Neuroptera	
Hymenoptera*	
Formicidae	
Diptera	
Lepidoptera	
Lepidoptera	

*Hymenoptera without Formicidae

Table 8 Number of individuals of foliage dwelling arthropod (Plot 3)

Number of individuals per sampling day (foliage dwellers)													
-1	+0	+2	+3	+4	+5	+7	+9	+11	+14	+21
2-5	2-5	6-10	2-5	6-10	6-10	6-10	11-25	6-10	11-25	11-25
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	1	1
1	-	26-50	6-10	51-100	26-50	51-100	51-101	51-100	51-100	51-100
-	-	1	1	-	1	-	1	-	-	2-5
-	2-5	6-10	6-10	6-10	6-10	6-10	11-25	6-10	11-25	26-50
-	-	-	-	-	-	-	-	-	-	-
1	-	2-5	2-5	11-25	1-5	6-10	1-25	2-5	51-100	6-10
11-25	11-25	6-10	6-10	11-25	11-25	11-25	26-50	-	-	-
6-10	26-50	11-25	11-25	11-25	26-50	26-50	26-50	26-50	26-50	26-50
-	-	-	-	-	-	-	-	-	-	-
6-10	6-10	26-50	11-25	11-25	1-25	1-25	1-25	6-10	11-25	26-50
11-25	11-25	6-10	6-10	11-25	11-25	6-10	6-10	2-5	51-100	11-25
2-5	2-5	6-10	2-5	6-10	6-10	6-10	6-10	6-10	26-50	11-25
1	-	-	1	-	1	101-200	-	2-5	-	6-10
11-25	26-50	26-50	26-50	26-50	51-100	26-50	26-50	51-100	51-100	51-100
-	-	-	2-5	1	1	2-5	6-10	11-25	26-50	26-50
1	-	-	-	-	-	-	-	-	-	-

Plot 3	
Taxonomic group	
Arachnida	
Isopoda	
Myriapoda	
Ephemeroptera	
Odonata	
Saltatoria	
Dermaptera	
Heteroptera	
Aphidina	
Coleoptera	
Coleoptera larvae	
Neuroptera	
Neuroptera larvae	
Hymenoptera*	
Formicidae	
Diptera	
Lepidoptera	
Lepidoptera larvae	

*Hymenoptera without Formicidae

Table 9 Number of individuals of foliage dwelling arthropods (Plot 4)

Number of individuals per sampling day (foliage dwellers)													
-1	+0	+1	+2	+3	+4	+5	+7	+9	+11	+14	+21
11-25	6-10	6-10	6-10	6-10	11-25	2-5	11-25	11-25	6-10	26-50	6-10
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	1	1
6-10	11-25	6-10	26-50	26-50	51-100	26-50	26-50	11-25	51-100	51-100	51-100
2-5	2-5	-	1	-	2-5	-	2-5	1	-	1	1
2-5	-	-	6-10	6-10	2-5	6-10	11-25	6-10	6-10	26-50	26-50
-	-	-	-	-	-	2-5	1	-	1	-	-
2-5	-	1	2-5	2-5	2-5	1	6-10	-	6-10	11-25	2-5
11-25	6-10	6-10	11-25	11-25	2-5	11-25	6-10	26-50	-	-	-
11-25	11-25	11-25	11-25	6-10	11-25	6-10	26-50	26-50	6-10	26-50	26-50
-	-	-	-	-	-	-	-	-	-	-	-
6-10	6-10	6-10	11-25	6-10	11-25	11-25	6-10	11-25	6-10	11-25	6-10
26-50	-	11-25	6-10	2-5	11-25	6-10	6-10	6-10	6-10	26-50	2-5
11-25	2-5	2-5	11-25	2-5	2-5	6-10	6-10	6-10	11-25	11-25	11-25
-	-	2-5	-	-	-	6-10	-	6-10	-	6-10	-
11-25	26-50	51-100	26-50	26-50	26-50	26-50	101-200	51-100	51-100	51-100	101-200
-	2	-	-	-	-	2-5	6-10	11-25	26-50	11-25	26-50
-	-	-	-	-	-	-	-	-	-	-	-

Plot 4	
Taxonomic group	
Arachnida	
Isopoda	
Myriapoda	
Ephemeroptera	
Odonata	
Saltatoria	
Dermaptera	
Heteroptera	
Aphidina	
Coleoptera	
Coleoptera larvae	
Neuroptera	
Neuroptera larvae	
Hymenoptera*	
Formicidae	
Diptera	
Lepidoptera	
Lepidoptera larvae	

*Hymenoptera without Formicidae

Results of residues of BYI 08330 and its metabolites detected in arthropods after application of the test substance are summarised in Table 10 to 17.

Table 10 Residues of BYI 08330 in ground dwellers

Sampling date	Residues [mg a.s./kg f.w.]					
	Orchard 1		Orchard 2		Mean	SD
	Plot 1	Plot 2	Plot 3	Plot 4		
-1	< LOQ	< LOQ	< LOQ	< LOQ	0.01	-
0 (before application)	0.08	< LOQ	< LOQ	0.08	0.05	-
+1	0.17	0.15	0.18	0.34	0.21	0.09
+2	0.25	0.21	0.36	0.44	0.32	0.10
+3	0.20	0.18	0.19	0.05	0.15	0.07
+4	0.36	0.30	0.15	0.41	0.31	0.11
+5	0.26	0.19	0.19	0.13	0.19	0.05
+6*	0.29	0.14	0.16	0.14	0.18	0.07
+7	0.32	0.08	0.13	0.14	0.17	0.11
+8*	0.24	0.09	0.14	0.14	0.15	0.06
+9	0.16	0.10	0.14	0.13	0.13	0.03
+10*	0.16	0.13	0.22	0.17	0.17	0.04
+11	0.16	0.15	0.29	0.21	0.20	0.06
+12*	0.15	0.12	0.22	0.19	0.17	0.04
+13*	0.15	0.09	0.14	0.17	0.14	0.03
+14	0.14	0.06	0.07	0.15	0.10	0.05
+15*	0.13	0.06	0.07	0.13	0.10	0.04
+16*	0.11	0.05	0.06	0.11	0.09	0.03
+17*	0.10	0.05	0.06	0.09	0.08	0.03
+18*	0.09	0.04	0.06	0.08	0.07	0.02
+19*	0.08	0.04	0.06	0.06	0.06	0.02
+20*	0.06	0.03	0.05	0.04	0.05	0.01
+21	0.05	0.03	0.05	0.02	0.04	0.02

*calculated data by linear interpolation; SD=standard deviation
< LOQ = 0.01 mg a.s./kg f.w.

Table 11 Residues of BYI 08330+enol in ground dwellers

Sampling date	Residues [mg/kg f.w.]					
	Orchard 1		Orchard 2		Mean	SD
	Plot 1	Plot 2	Plot 3	Plot 4		
-1	< LOQ	0.02	< LOQ	< LOQ	0.02	-
0 (before application)	0.11	< LOQ	< LOQ	0.11	0.07	-
+1	0.42	0.29	0.44	0.94	0.52	0.29
+2	0.68	0.32	0.80	0.60	0.60	0.20
+3	0.34	0.33	0.62	0.09	0.35	0.22
+4	0.59	0.39	0.25	0.79	0.51	0.24
+5	0.46	0.47	0.37	0.27	0.39	0.09
+6*	0.50	0.31	0.55	0.29	0.41	0.13
+7	0.54	0.13	0.72	0.31	0.43	0.25
+8*	0.45	0.17	0.55	0.54	0.43	0.18
+9	0.35	0.19	0.37	0.77	0.42	0.25
+10*	0.32	0.24	0.79	0.63	0.50	0.26
+11	0.29	0.28	1.2	0.48	0.57	0.44
+12*	0.26	0.25	0.89	0.41	0.46	0.30
+13*	0.26	0.21	0.56	0.34	0.35	0.15
+14	0.25	0.18	0.24	0.25	0.24	0.04
+15*	0.23	0.17	0.24	0.25	0.22	0.04
+16*	0.21	0.16	0.24	0.22	0.21	0.04
+17*	0.19	0.15	0.24	0.20	0.20	0.04
+18*	0.16	0.14	0.25	0.18	0.18	0.05
+19*	0.14	0.13	0.25	0.16	0.17	0.05
+20*	0.12	0.12	0.25	0.13	0.16	0.06
+21	0.10	0.11	0.25	0.11	0.14	0.07

*calculated data by linear interpolation
< LOQ cal. = 0.022 mg/kg f.w.

Table 12 Total measured residues value in ground dwellers

Sampling date	Residues [mg/kg f.w.]					
	Orchard 1		Orchard 2		Mean	SD
	Plot 1	Plot 2	Plot 3	Plot 4		
-1	< LOQ	< LOQ	< LOQ	< LOQ	0.03	-
0 (before application)	0.11	< LOQ	< LOQ	0.11	0.08	-
+1	0.44	0.31	0.45	0.95	0.54	0.28
+2	0.71	0.34	0.82	0.62	0.62	0.21
+3	0.37	0.37	0.64	0.10	0.37	0.22
+4	0.62	0.42	0.25	0.81	0.53	0.24
+5	0.50	0.50	0.39	0.28	0.42	0.11
+6*	0.54	0.34	0.62	0.31	0.45	0.15
+7	0.57	0.18	0.85	0.34	0.48	0.29
+8*	0.48	0.20	0.64	0.58	0.47	0.19
+9	0.39	0.21	0.45	0.81	0.46	0.25
+10*	0.36	0.27	0.86	0.67	0.54	0.28
+11	0.32	0.32	1.30	0.53	0.61	0.47
+12*	0.31	0.28	0.96	0.46	0.50	0.32
+13*	0.30	0.25	0.63	0.38	0.39	0.17
+14	0.29	0.21	0.29	0.34	0.27	0.04
+15*	0.27	0.20	0.29	0.29	0.27	0.04
+16*	0.25	0.20	0.30	0.28	0.26	0.04
+17*	0.23	0.19	0.30	0.26	0.25	0.05
+18*	0.20	0.19	0.31	0.24	0.24	0.05
+19*	0.18	0.18	0.31	0.22	0.23	0.06
+20*	0.16	0.18	0.32	0.21	0.22	0.07
+21	0.14	0.17	0.32	0.19	0.21	0.08

*calculated data by linear interpolation, SD= standard deviation
< LOQ cal. = 0.034 mg/kg f.w.

Table 13 Residues of BYI 08330 in foliage dwellers

Sampling date	Residues [mg a.s./kg f.w.]					
	Orchard 1		Orchard 2		Mean	SD
	Plot 1	Plot 2	Plot 3	Plot 4		
-1	< LOQ	< LOQ	< LOQ	0.03	0.01	
0 (after application)	3.4	4.5	4.1	3.8	4.0	0.47
+1	4.4	1.6	4.5	4.9	3.9	1.5
+2	2.2	1.1	3.4	2.9	2.4	1.0
+3	0.71	1.3	1.7	1.6	1.3	0.45
+4	0.99	0.71	2.2	1.1	1.2	0.63
+5	0.97	1.7	1.3	0.94	1.2	0.35
+6*	0.93	1.3	1.2	0.91	1.1	0.19
+7	0.88	0.89	1.1	0.88	0.95	0.11
+8*	0.82	0.8	0.9	1.1	0.90	0.17
+9	0.75	0.68	0.61	1.4	0.84	0.37
+10*	0.65	0.59	0.66	1.0	0.73	0.20
+11	0.54	0.53	0.70	0.64	0.60	0.08
+12*	0.53	0.53	0.63	0.56	0.61	0.09
+13*	0.51	0.91	0.57	0.48	0.62	0.20
+14	0.50	1.1	0.50	0.40	0.63	0.32
+15*	0.45	0.97	0.46	0.36	0.56	0.28
+16*	0.40	0.83	0.41	0.31	0.49	0.23
+17*	0.35	0.70	0.37	0.27	0.42	0.19
+18*	0.29	0.56	0.33	0.23	0.35	0.15
+19*	0.24	0.43	0.29	0.19	0.29	0.10
+20*	0.19	0.29	0.24	0.14	0.22	0.07
+21	0.14	0.16	0.20	0.10	0.15	0.04

*calculated data by linear interpolation; SD=standard deviation
< LOQ = 0.01 mg a.s./kg f.w.

Table 14 Residues of BYI 08330+enol in foliage dwellers

Sampling date	Residues [mg/kg f.w.]					
	Orchard 1		Orchard 2		Mean	SD
	Plot 1	Plot 2	Plot 3	Plot 4		
-1	0.02	0.02	0.02	0.06	0.03	0.02
0 (after application)	4.9	5.6	5.2	5.0	5.2	0.31
+1	6.4	4.0	6.8	6.7	6.0	1.3
+2	3.5	2.0	4.3	4.0	3.7	1.3
+3	2.5	2.1	2.8	2.8	2.5	0.33
+4	1.8	2.1	3.3	2.2	2.3	0.67
+5	2.0	2.4	3.3	2.4	2.5	0.56
+6*	2.4	1.9	2.6	2.0	2.2	0.34
+7	2.8	1.4	1.9	1.6	1.7	0.61
+8*	2.1	1.8	2.2	2.2	2.1	0.20
+9	1.4	2.2	2.5	2.8	2.2	0.59
+10*	1.2	1.6	2.1	2.1	1.7	0.43
+11	0.99	0.99	1.7	1.4	1.3	0.33
+12*	0.96	1.1	1.4	1.2	1.2	0.18
+13*	0.92	1.3	1.1	0.99	1.1	0.17
+14	0.88	1.4	0.89	0.80	1.0	0.29
+15*	0.85	1.3	0.84	0.77	0.94	0.24
+16*	0.81	1.2	0.79	0.73	0.87	0.19
+17*	0.78	1.0	0.74	0.70	0.81	0.14
+18*	0.74	0.87	0.68	0.66	0.74	0.10
+19*	0.71	0.73	0.63	0.63	0.68	0.053
+20*	0.67	0.59	0.58	0.59	0.61	0.043
+21	0.64	0.45	0.53	0.56	0.55	0.079

*calculated data by linear interpolation

Table 15 Total measured residue values in foliage dwellers

Sampling date	Residues [mg/kg f.w.]					
	Orchard 1		Orchard 2		Mean	SD
	Plot 1	Plot 2	Plot 3	Plot 4		
-1	< LOQ	< LOQ	< LOQ	0.06	0.04	
0 (after application)	5.1	5.7	5.4	5.0	5.3	0.32
+1	6.7	4.1	7.0	6.8	6.2	1.37
+2	3.7	2.1	4.5	3.1	3.8	1.30
+3	2.8	2.1	2.8	2.8	2.6	0.35
+4	1.9	2.2	3.3	2.3	2.4	0.61
+5	2.2	2.5	3.5	2.5	2.7	0.57
+6*	2.3	2.0	2.8	2.1	2.3	0.36
+7	2.3	1.5	2.1	1.7	1.9	0.37
+8*	1.9	1.9	2.5	2.3	2.1	0.28
+9	1.5	2.3	2.8	2.9	2.4	0.64
+10*	1.3	1.7	2.3	2.3	1.9	0.48
+11	1.1	1.0	1.8	1.6	1.4	0.39
+12*	1.1	1.2	1.5	1.4	1.3	0.20
+13*	1.0	1.4	1.2	1.1	1.2	0.17
+14	0.96	1.6	0.97	0.86	1.1	0.34
+15*	0.94	1.4	0.92	0.85	1.0	0.27
+16*	0.93	1.3	0.87	0.85	0.98	0.21
+17*	0.91	1.1	0.82	0.84	0.93	0.15
+18*	0.90	0.99	0.76	0.84	0.87	0.09
+19*	0.88	0.84	0.71	0.83	0.82	0.07
+20*	0.87	0.68	0.66	0.83	0.76	0.10
+21	0.85	0.53	0.61	0.82	0.70	0.16

*calculated data by linear interpolation; SD=standard deviation
< LOQ cal. = 0.034 mg/kg f.w.

Table 16 Calculation of the TWAs for ground dwellers

Maximum TWA for daily periods [mg/kg f.w.]								
Parent			**Parent+Enol**			**Total measured residue**		
Period [d] (DAT)	[mg a.s./ kg f.w.]	% of MMV	Period [d] (DAT)	[mg /kg f.w.]	% of MMV	Period [d] (DAT)	[mg/kg f.w.]	% of MMV
1 (1-2)	0.26	83	1 (1-2)	0.56	93	1 (1-2)	0.58	93
2 (2-4)	0.26	81	2 (10-12)	0.51	84	2 (10-12)	0.55	88
3 (1-4)	0.25	78	3 (1-4)	0.50	83	3 (9-12)	0.53	85
4 (1-5)	0.23	74	4 (1-5/8-12)	0.47	79	4 (8-12)	0.52	83
5 (1-6)	0.23	72	5 (7-12)	0.47	78	5 (7-12)	0.51	82
6 (1-7)	0.22	69	6 (1-7/6-12)	0.46	76	6 (6-12)	0.50	81
7 (1-8)	0.21	66	7 (4-11)	0.46	76	7 (4-11/5-12)	0.49	79
8 (1-9)	0.20	64	8 (4-12)	0.46	76	8 (4-12)	0.49	79
9 (1-10)	0.20	63	9 (2-11)	0.46	77	9 (2-11)	0.49	79
10 (1-11)	0.20	63	10 (1-11)	0.47	78	10 (1-11/2-12)	0.50	80
11 (1-12)	0.20	62	11 (1-12)	0.46	77	11 (1-12)	0.50	80
12 (1-13)	0.19	61	12 (1-13)	0.46	76	12 (1-13)	0.49	79
13 (1-14)	0.18	59	13 (1-14)	0.44	73	13 (1-14)	0.47	76
14 (1-15)	0.18	57	14 (1-15)	0.43	71	14 (1-15)	0.46	74
15 (1-16)	0.17	55	15 (1-16)	0.41	69	15 (1-16)	0.45	72
16 (1-17)	0.17	53	16 (1-17)	0.40	67	16 (1-17)	0.44	70
17 (1-18)	0.16	51	17 (1-18)	0.39	65	17 (1-18)	0.42	68
18 (1-19)	0.16	49	18 (1-19)	0.38	63	18 (1-19)	0.41	67
19 (1-20)	0.15	48	19 (1-20)	0.37	61	19 (1-20)	0.40	65
20 (1-21)	0.15	46	20 (1-21)	0.35	59	20 (1-21)	0.39	63

MMV= Maximum Measured Value

Table 17 Calculation of the maximum TWAs for foliage dwellers

Maximum TWA for daily periods [mg/kg f.w.]								
Parent			Parent+Enol			Total measured residue		
Period [d] (DAT)*	[mg a.s./ kg f.w.]	% of MMV	Period [d] (DAT)*	[mg /kg f.w.]	% of MMV	Period [d] (DAT)*	[mg/kg f.w.]	% of MMV
1 (0-1)	3.90	99	1 (0-1)	5.59	93	1 (0-1)	5.73	93
2 (0-2)	3.40	86	2 (0-2)	4.96	83	2 (0-2)	5.08	83
3 (0-3)	2.88	73	3 (+0-3)	4.34	72	3 (0-3)	4.47	73
4 (0-4)	2.56	65	4 (0-4)	3.94	66	4 (0-4)	4.06	66
5 (0-5)	2.33	59	5 (0-5)	3.70	62	5 (0-5)	3.83	62
6 (0-6)	2.16	55	6 (0-6)	3.49	58	6 (0-6)	3.61	59
7 (0-7)	2.00	51	7 (0-7)	3.26	54	7 (0-7)	3.40	55
8 (0-8)	1.88	48	8 (0-8)	3.13	52	8 (0-8)	3.26	53
9 (0-9)	1.78	45	9 (0-9)	3.04	51	9 (0-9)	3.17	52
10 (0-10)	1.68	43	10 (0-10)	2.92	49	10 (0-10)	3.05	50
11 (0-11)	1.59	40	11 (0-11)	2.78	46	11 (0-11)	2.91	47
12 (0-12)	1.52	38	12 (0-12)	2.66	44	12 (0-12)	2.79	45
13 (0-13)	1.45	37	13 (0-13)	2.55	42	13 (0-13)	2.67	43
14 (0-14)	1.40	35	14 (0-14)	2.44	41	14 (0-14)	2.57	42
15 (0-15)	1.34	34	15 (0-15)	2.35	39	15 (0-15)	2.47	40
16 (0-16)	1.29	33	16 (0-16)	2.26	38	16 (0-16)	2.38	39
17 (0-17)	1.25	32	17 (0-17)	2.18	36	17 (0-17)	2.30	37
18 (0-18)	1.20	30	18 (0-18)	2.11	35	18 (0-18)	2.23	36
19 (0-19)	1.15	29	19 (0-19)	2.03	34	19 (0-19)	2.16	35
20 (0-20)	1.11	28	20 (0-20)	1.97	33	20 (0-20)	2.09	34
21 (0-21)	1.07	27	21 (0-21)	1.90	32	21 (0-21)	2.03	33

MMV: Maximum measured value
*on DAT 0 inventory spraying was carried out after application

B. Observations

Arachnida, Isopoda, Myriapoda, Coleoptera, Dermaptera and Lepidoptera larvae were the most abundant groups in the pitfall traps, whereas Ephemeroptera, Diptera, Coleoptera, Neuroptera

(adults and larvae) and Hymenoptera (without Formicidae) were the most abundant arthropod groups in the samples of inventory spraying (see Table 2 to 9).

Ground dwellers

The initial measured values (IMV) of the mean residues (n = 4; DAT +1) in ground dwelling arthropods were 0.21, 0.52 and 0.54 mg/kg f.w. for parent, parent+enol and total measured residue, respectively (see Table 10 to 12).

The maximum measured values (MMV) of the mean residues (n = 4; DAT +2) in ground dwelling arthropods were 0.32, 0.60 and 0.62 mg/kg f.w. for parent, parent+enol and total measured residue, respectively.

TWAs of parent, parent+enol and total measured residue were calculated for the whole study period (DAT +1-21) with 0.15 (46% of MMV), 0.35 (59% of MMV) and 0.39 mg a.s./kg f.w. (63% of MMV), respectively. For all maximum TWAs of daily periods see Table 16.

Foliage dwellers

IMV of the mean residues (n = 4; DAT +0) in foliage dwelling arthropods were 4.0, 5.2 and 5.3 mg/kg f.w. for parent, parent+enol and total measured residue, respectively (see Table 13 to 15).

MMV of the mean residues (n = 4) in foliage dwelling arthropods were 4.0 (DAT +0), 6.0 (DAT +1) and 6.2 mg/kg f.w. (DAT +1) for parent, parent+enol and total measured residue, respectively.

TWAs of parent, parent+enol and total measured residue were observed for the whole study period (DAT +0-21) with 1.07 (27% of MMV), 1.90 (32% of MMV) and 2.03 mg/kg f.w. (33% of MMV), respectively. All maximum TWAs of daily periods are shown in Table 17.

The DT_{50} of parent, parent+enol and total measured residue in foliage dwellers followed a first-order kinetic. The DT_{50} for residue dissipation was 2.8, 4.7 and 5.1 days for the parent, parent+enol and total measured residue, respectively. The fitted curves of degradation of parent, parent+enol and total measured residue are shown in the Analytical Phase Report attached to the original study report.

CONCLUSION

The study provides realistic data on magnitude of peak residue levels and the corresponding time course of residue decline of BYI 08330 and its metabolites BYI 08330-enol and BYI 08330-ketohydroxy in ground and foliage dwelling arthropods. Maximum measured residues in ground dwellers were 0.32, 0.60 and 0.62 mg/kg f.w. for parent, parent+enol and total measured residues, respectively. In foliage dwellers maximum measured residues were ten times higher than in ground dwellers, with 4.0, 6.0 and 6.2 mg/kg f.w. for parent, parent+enol and total measured residues, respectively. The decline of the residue levels of parent, parent+enol and total measured residue in foliage dwellers followed a first-order kinetic. The DT_{50} for residue dissipation was 2.8, 4.7 and 5.1 days for parent, parent+enol and total measured residue, respectively. These data provide a reliable basis for use in higher tier risk assessments of insectivorous and omnivorous birds and mammals.

IIA 8.17 Summary and evaluation of points IIA 7 and IIA 8.1 to 8.16

Overall summary: IIA7, Fate and behavior in the environment

Fate and behavior in soil

The basic soil metabolism study showed that parent compound BYI08330 is quickly degraded. Already after 1-2 days more than 90% of the test item dissipated and declined. At study termination, evolved $^{14}CO_2$ (no volatile organics occurred) accounted for up to 19.4% of AR at DAT-50 (EU soils), and accounted for 15.3% of AR for the US soil after 360 days. During the course of the study a number of degradates was observed in all four soils. Besides the two main soil metabolites BYI08330-enol (max. 24.3% of AR at DAT-3) and BYI08330-ketohydroxy (max. 16.3%, DAT-1), two enol-dimers (more or less artificially formed) and BYI08330-MA-amide (max. 6.4%, DAT-179; were identified. In addition, two minor degradates were identified as BYI08330-desmethyl-enol and BYI08330-oxo-enol amounting to maximum 3.7% and 1.2% of AR, respectively.

Furthermore, the biotransformation of spirotetramat was investigated in two soils using [azaspirodecenyl-3-^{14}C]BYI08330 for 127 days under outdoor climatic conditions realistic for the intended use. Thereby BYI08330 formulated as an OD 400 (pH 5) was applied at 94.6% of the highest recommended single use rate for field application (288 g/ha). The parent compound was quickly and thoroughly degraded, and already one day after application, only 52.6 and 72.2% of the applied test item were detectable in both soils. During the course of the study a number of degradates was observed in all four soils. Only two major degradates were detected, BYI08330-ketohydroxy (max. 25.3% AR, DAT-14) and BYI08330-enol (max. 7.8% AR, DAT-7). Three minor metabolites were identified as glyoxylic amide, benzoic acid and ketohydroxy-carboxy. The results obtained confirmed and completed the pathway already established in the guideline aerobic soil metabolism laboratory studies.
In the BYI08330 studies the soil processing procedure was optimized to get >90% extraction efficiency and >90% recovery of the test item at time zero. However, under the acidic extraction conditions needed for spirotetramat, the major metabolite BYI08330-enol was found to be partly unstable. It degrades under the formation of BYI08330-ketohydroxy and others. Therefore, the degradation and metabolism of the BYI08330-enol in soil was investigated in a separate study (see below), and those results needed to be included in the proposed overall metabolic pathway of spirotetramat in soil. This fact was also the reason to base the degradation kinetics of the major spirotetramat metabolites on the BYI08330-enol study, but not on the parent study

Thus, the biotransformation of [azaspirodecenyl-3-^{14}C] and [azaspiro-decenyl-5-^{14}C]BYI08330-enol was studied in three EU soils and one US soil for 119 days under aerobic conditions in the dark at 20 ±1 °C and at approx. 80% of 1/3 bar moisture (US soil) or 60% WHC_{max} (EU soils). BYI08330-enol dissipated following pronounced biphasic kinetics, with an extremely quick first phase. Within a second slower degradation phase, the test item declined to 2.7 to 6.1% of AR in the four soils at the end of the study at DAT-119. During the course of the study a number of degradates was observed in all four soils. Label-specific degradates were not observed throughout the entire study, and the degradation pathway found in before-mentioned study on spirotetramat was confirmed. In addition, a metabolite previously not found, the BYI08330-oxo-ketohydroxy was identified. However, it was a very minor contaminant and was not quantifiable. In all four soils, BYI08330-ketohydroxy was analyzed at levels > 10%. BYI08330-enol-dimer 1 amounted once to 5.0% (DAT-60) in one soil, and BYI08330-enol-dimer 2 to a maximum of 3.6% (DAT-

14). BYI08330-MA-amide amounted once >5% (5.2% at DAT-4), and BYI08330-desmethyl-enol was max. 1.8% at DAT-4. All degradates were transient during the study and did not increase towards the end of the study.

Based on the degradate profiles obtained within an anaerobic soil metabolism study, a degradation pathway was proposed which is almost identical to the degradation pathway obtained in aerobic soil. It is concluded that BYI08330 applied to soil will be degraded rapidly in a subsequently flooded anaerobic soil situation, and will not form degradates different from those observed in soil under aerobic conditions, and/or known from abiotic hydrolysis experiments (see section later).

The parent compound was well degraded on irradiated soil samples of phototransformation study on soil surface. However, the biotransformation in the dark controls was approx. four times faster and, considering real environmental conditions (e.g. in June at Athens, Greece) even approx. 20 times faster compared to soil samples irradiated by natural sunlight. This kinetics results together with the findings that the pathway of degradation was similar indicate that a distinct phototransformation product is not to be expected in soil after the use of spirotetramat under outdoor conditions.

Referring to the behavior in the environment it can be concluded that the active substance spirotetramat (BYI08330) predominantly degrades to the metabolite BYI08330-enol which is further oxidized to BYI08330-ketohydroxy. Subsequently BYI08330-ketohydroxy is hydrolytically opened to BYI8330-MA-amide, as it is included in the proposed overall metabolic scheme outlined in **Fehler! Verweisquelle konnte nicht gefunden werden.**. All components are subject to further degradation to form non-extractable residues (NER) and CO_2.

The found normalized geometric mean BYI08330 DT_{50} value of 0.13 days is a suitable input parameter for environmental fate models. Further, the kinetics of biotransformation of spirotetramat was investigated in two soils using ^{14}C-BYI08330 for 127 days under outdoor climatic conditions realistic for the intended use. Thereby ^{14}C-BYI08330 formulated as an OD 100 (pH 5) was applied at 94.6% of the highest recommended single use rate for field application (288 g/ha). The parent compound was quickly and thoroughly degraded, and a mean DT_{50} of approx. 2 days was estimated.

The investigation of BYI08330-enol as test item showed that it dissipates following a biphasic kinetics, with an extremely quick first phase. This portion is regarded as a strong bound fraction of BYI08330-enol in soil. The respective kinetic modeling of test item by using MatLab® (application KinGUI) indicated that the best fit DT_{50} (days) of test item resulted by using the bi-exponential model DFOP (double first order in parallel). This model yielded a mean BYI08330-enol DT_{50} value of 0.08 days (chi^2 statistics mean value of 7.7). Thus it can be concluded that BYI08330-enol is a fast degrading major metabolite of spirotetramat in soil.
A more detailed kinetics modeling investigation based on the results of BYI08330 and BYI08330-enol studies yielded geometric mean normalized $DT_{50\text{-}ref}$ values of 0.13 days for BYI08330-enol, 3.8 days for BYI08330-ketohydroxy and 1 day for BYI08330-MA-amide. They are suitable input parameters for environmental fate models. It must be noted, that the extremely short half life time of 0.13 days for BYI08330-enol may only be used for modeling purposes and in conjunction with the SFORB model or a kinetic sorption model as implemented in PEARL.

Based on the results obtained within a further laboratory soil degradation study using three

aerobic soils it was shown that the metabolite 4-methoxy cyclohexanone is fast and steadily degrading in soil (DT_{50} < 1 day) and that there is no potential for accumulation of 4-methoxy cyclohexanone residues in viable soils. The observed higher level of 4-methoxy cyclohexanone residues in the laboratory study on phototransformation of BYI08330 on soil surface might have been caused by a decreasing viability of test soil during the strong irradiation in such a laboratory test system.

From an anaerobic soil metabolism study it is concluded that BYI08330 applied to soil will be degraded rapidly in a flooded anaerobic soil situation and will not form degradates different from those observed in soil under aerobic conditions, and/or known from abiotic hydrolysis experiments. Compared to the before mentioned fast biotransformation in dark soils (DT_{50} < 1 day) phototransformation of BYI09330 on soil surface is not regarded as a relevant degradation process under environmental sunlight irradiation conditions.

From all the laboratory studies and a radiolabeled outdoor study it can be concluded that spirotetramat is a very fast degrading compound in soil, and all metabolites generated from BYI08330-enol, the predominant first metabolite, are further degraded and are expected not to accumulate in the environment. The soil dissipation testing in a range of representative soils and locations in the USA confirmed that findings.

In order to determine the residues during US terrestrial field dissipation trials an analytical method (FN-002-S05-02) for the determination of BYI08330 and its metabolites BYI08330-enol, BYI08330-ketohydroxy, and BYI08330-MA-amide in soil and sediment by LC/MS/MS was developed and successfully validated for the determination of residues in soil and sediment. The method was evaluated by determining the average recoveries and relative standard deviation at the LOQ of 5 ng/g and at 5x LOQ (25 ng/g).

BYI08330 dissipated rapidly in soil under field conditions. The dissipation rates of BYI08330 calculated for four sites in the US, resulted in half-life (DT_{50}) values from 0.9 to 1.0 days and the periods required for 90% dissipation (DT_{90}) ranged 1.1 to 3.5 days with no apparent obvious correlation with soil properties or the management (bare ground Vs. cropped). The DT_{50} values of the combined residues of BYI08330 (i.e. BYI08330, BYI08330-enol, and BYI08330-ketohydroxy) ranged from 5.0 to 23.4 days and the DT90 values ranged from 16.7 to 77.8 days. Residues of BYI08330 did not move below the surface layer (0 to 15 cm) in all the sites, except in Florida where residues of BYI08330-enol and BYI08330-ketohydroxy were detected above the LOQ at 15 to 30 cm layer between 1 day and 7 days after application. After that the residues completed degradation to less than LOQ and MDL. It should be noted that the Florida site represents a worst case condition with heavy rainfall and very light soil (95% sand in the surface layer) with very low organic matter (0.5%). Therefore, leaching and groundwater contamination is not likely with BYI08330.

BYI08330 degraded to less than the MDL levels (0.5 µg/kg) within 14 days after application. The soil concentration the metabolites of BYI08330 were below the LOQ within 28 to 365 days after application. Based on these results, the carry over potential of soil residues from one year to another is very low.

Considering the results from laboratory soil metabolism studies and terrestrial field dissipation studies the major route(s) of dissipation for BYI08330 are degradation to BYI08330-enol and BYI08330-ketohydroxy, subsequent biodegradation to non-extractable soil residues and mineralization to CO_2.

BYI08330 and its metabolites BYI08330-ketohydroxy and BYI08330-MA-amide showed no evidence of any degradation in the four soils during a maximum storage interval of 334 days in frozen storage and there was little variation in the results for the four soils. BYI08330-enol recoveries declined during storage, with the majority of the loss occurring during the first 30 days of storage. The primary causes of these low recoveries were degradation of BYI08330-enol to BYI08330-ketohydroxy and binding of the analyte to soil.

Mobility

Freundlich adsorption and desorption constants K_F and K_{OC} of spirotetramat have been determined in batch equilibrium experiments with five different soils using radiolabeled test substance ([azaspirodecenyl-3-^{14}C]BYI08330). Since significant degradation of test item was observed in a pre-test, the main test was performed with sterilized soil. K_{OC} values for the different soils were in the range of 159 to 435 mL/g with a mean K_{OC} of 281 mL/g (1/n = 0.941). Based on this value, spirotetramat can be classified as low mobile in soil.

Freundlich adsorption and desorption constants K_F and K_{OC} of BYI08330-enol, the major metabolite in soil, was attempted in batch equilibrium experiments with five different soils using radiolabeled test substance ([azaspirodecenyl-3-^{14}C]BYI08330-enol. However, the study showed that the sorption characteristics of BYI08330-enol to soil cannot be determined by a batch equilibrium test according to OECD Guideline 106. In order to asses the environmental behavior of the test item more suitable test methods had to be employed. Another option, i.e. a so-called time-dependent sorption study, demonstrated that sorption and binding of BYI08330-enol to soil is extremely fast and increases very rapidly with aging time in soil. The portion not tightly bound to soil, i.e. the portion that is releasable by aqueous solution from soil (weakly sorbed), is degraded within a few hours. From these results it can be concluded that BYI08330-enol is absent from the soil pore water (either degraded or tightly bound to soil) within a very short period of time This study confirmed that the sorption characteristics of the test item BYI08330-enol to soil cannot be determined accurately by a batch equilibrium test according to OECD TG 106. Since only partial degradation of BYI08330-enol occurred during the course of a soil column leaching study performed with four soils the test system allowed the calculation of adsorption constants for the test item in soils. For the strongly bound BYI08330-enol fraction K_{OC} values between 828 and 1711 mL/g were calculated, resulting in a mean value of 1187 mL/g over four soil types. For the weakly bound BYI08330-enol fraction K_{OC} values between 27 and ca. 99 mL/g were calculated, resulting in a mean value of 55 mL/g over four soil types. Based on the classification of soil mobility potential according to Briggs, the strongly sorbed BYI08330-enol fraction is classified as immobile, and the weakly bound BYI08330-enol fraction has an intermediate potential to leach through soil.

Freundlich adsorption and desorption constants K_F and K_{OC} of BYI08330-ketohydroxy, a major metabolite in soil, have been determined in batch equilibrium experiments with five different soils using [azaspirodecenyl-3-^{14}C]BYI08330-ketohydroxy. Since significant degradation of BYI08330-ketohydroxy was observed in a pre-test, the equilibration solution used was 0.01 M aqueous $CaCl_2$ solution spiked with 50 mg $HgCl_2$ as biocide. $K_{OC(ads)}$ values for the different soils

were in the range of 41.0 to 99.1 mL/g with a mean K_{OC} of 63.7 mL/g (1/n = 0.922). Based on this value, BYI08330-ketohydroxy can be classified as intermediate to mobile in soil.

Freundlich adsorption and desorption constants K_F and K_{OC} of BYI08330-MA-amide have been determined in batch equilibrium experiments with five different soils using [hydroxy-^{14}C]BYI08330-MA-amide. The calculated adsorption constants K_F of the Freundlich isotherms for the four test soils ranged from 0.06 to 0.18, and the $K_{OC(ads)}$ values were in the range of 4.4 to 25.5 mL/g with a mean $K_{OC(ads)}$ of 9.3 mL/g (mean 1/n = 0.948). Based on this value, BYI08330-MA-amide can be classified as high mobile in soil. The desorption K_{des} values were 0.13 to 0.37 and higher than those obtained for K_F in the adsorption phase indicating a little stronger binding once adsorbed to soil.

BYI08330-enol dimers 1 and 2 were minor metabolites in the relevant metabolism studies. Nevertheless, their adsorption coefficients on soil were estimated by using the HPLC method according to OECD TG No. 121. The soil adsorption coefficients of BYI08330-enol dimer 1 were estimated to be log K_{OC} = 3.23 and K_{OC} = 1695. For the BYI08330-enol dimer 2 a log K_{OC} = 3.46 and a K_{OC} = 2879 were estimated. According to the Briggs' classification BYI08330-enol dimer 1 and BYI08330-enol dimer 2 would be categorized as immobile.

From all the before mentioned laboratory studies it is concluded that the mobility of spirotetramat residues in soil is sufficiently understood. Since a long-term leaching simulations indicated that the PEC_{GW} values are generally far below 0.1 µg/L in all application scenarios relevant for the intended uses of spirotetramat on citrus, oranges, mandarins, lemons, limes, etc. in EU-South, and on lettuce in EU-North and EU-South, no concern with regard to groundwater contamination of parent compound and its metabolites is indicated, and a safe use is given in the EU.

Volatility

Based on the vapor pressure of BYI08330 of 5.6×10^{-9} Pa and BYI08330-enol5 of 1.2×10^{-10} Pa, considerable volatilization of both substances when applied to soil surfaces or leaves is not to be expected. This evaluation is also confirmed by the rating of trigger values for volatilization as described in the model EVA 2.0 developed from the FOCUS Air group. There, for compounds with a vapor pressure < 10^{-4} Pa at 20 °C volatilization from soil surfaces or with a vapor pressure < 10^{-5} Pa at 20 °C volatilization from plant surfaces is not considered relevant.

Fate and behavior in water

The fate and behavior of spirotetramat in aquatic systems was investigated under standardized laboratory conditions, using radiolabeled as well as unlabeled test substance. Under dark conditions spirotetramat was found to be degradable by abiotic degradation processes. Hydrolysis is regarded as relevant for the degradation of BYI08330 in the environment, especially under neutral and alkaline conditions. The hydrolytic half-life at pH 7 and 25°C (20°C) is expected to be in the range of 8.6 days (13 days). In the total pH range tested (pH 4 to 9) the formation of BYI08330-enol as the only common hydrolysis product was observed. From a separate study

5 : The vapor pressure was estimated by using MPBPWIN v1.41 (MPBPWIN™ is owned by the U.S. E PA): acc. to Modified Grain Method a vapor pressure of 0.912 x 10E-12 mm Hg at 20 °C resulted, then transferred to Pa by a factor of 133.3.

investigating the hydrolysis of the major degradate it was concluded that hydrolysis is not relevant for the degradation of BYI08330-enol in the environment, since the hydrolytic half-life at pH 4, 7 and 9 at 25°C is expected to be > 1 year.

Based on the experimental DT_{50} of 2.7 days for BYI08330 in sterile pure buffered water and related predicted environmental DT_{50} (e.g. of 12.9 solar summer days at Phoenix, AZ, USA or 19.9 summer days at Athens, Greece) it was concluded that photo-transformation of BYI08330 in aqueous systems is a significant route for the elimination of this compound. However, that basic tests are to be performed under sterile conditions in highly purified buffer of pH 5, in order to help distinguish between hydrolytic or/and biotic and direct photolytic reactions. Thus, it was expected that the behavior will be different in natural aqueous systems, since in the case of BYI08330 biodegradation will happen quickly, hydrolysis will be faster with increasing pH, as well as indirect reactions might compete with the re-arrangement reactions observed in the prevailing study. This expectations were confirmed by an investigation of the phototransformation of [^{14}C]BYI08330 (labels #1 and #2) in sterile natural water by a supportive study. Based on the experimental DT_{50} of 0.2 days for BYI08330 and related predicted environmental DT_{50} (e.g. of 0.6 solar summer days at Phoenix, AZ, USA or 1.0 summer days at Athens, Greece) it is concluded that photo-transformation of BYI08330 in aqueous systems is a significant route for the elimination of this compound in natural water. This test clearly showed that competition of hydrolysis and indirect photo-reactions does not allow the light-induced re-arrangement reactions of parent compound observed in highly purified buffer of pH 5. Together with the well-known fast biodegradation this was the justification to consider the products formed in the natural water study as relevant for the overall pathway of spirotetramat degradation in water, but not the re-arrangement photo-products found in the highly artificial laboratory study performed in sterile pure buffer.

From a laboratory study investigating the route and rate of degradation in two natural water/sediment systems under aerobic laboratory conditions in the dark at 20 °C it is concluded that BYI08330 once entering aqueous systems will be degraded rapidly and thoroughly, mainly via the metabolites BYI08330-enol and BYI08330-ketohydroxy. DT_{50} values of 1.00 and 1.02 days were calculated for BYI08330 in the water phase, and 1.06 and 1.05 days in case of the entire systems, respectively. For use as persistence endpoints conservative one compartment (Level I) SFO dissipation half-lives of BYI08330, BYI08330-enol and BYI08330-ketohydroxy were derived for the water and the sediment compartment in a special modeling study. The biphasic model FOMC model was tested, though did not improve the outcome for any of the compounds. SFO total system degradation half-lives were derived to be used as modeling endpoints for both compartments at FOCUS surface water STEP 2 level and STEP 3 level.

The results of a laboratory study investigating the route and rate of degradation in a completely anaerobic water/sediment system in the dark at 20 °C showed that BYI08330 once entering an anaerobic natural aqueous environment will also be degraded rapidly, mainly to the metabolite BYI08330-enol well known from the studies in aerobic soil and water/sediment systems, already. The first-order degradation rate calculated for BYI08330 in anaerobic water, sediment, and in the entire system resulted in half-lives of 2.8, 3.1, and 2.8 days, respectively.

Fate and behavior in air

Reactions with OH radicals and ozone contribute to the degradation of BYI08330 and BYI08330-enol in the air to a high extent. The chemical stabilities in air are not determined by an attack at one single site, but at different parts of the molecule. This should result in the formation of various primary radicals leading to secondary oxidation products, which can be eliminated from the air by wet and/or dry deposition.
On account of an estimated chemical lifetime of both compounds in the air of at the most 3 hours it is to be expected that they can not be transported in gaseous phase over large distances and can not accumulate in the air.

Overall summary: IIA8.1 – 8.16, Ecotoxicological Studies

Effects on birds

Acute toxicity to birds	LD_{50} > 2000 mg a.s./kg b.w. (Bobwhite quail) LD_{50} > 2000 mg a.s./kg b.w. (Chicken) LD_{50} > 2000 mg a.s./kg b.w. (Canary) LD_{50} > 2000 mg product/kg b.w. (Chicken; OD 150) LD_{50} > 2000 mg product/kg b.w. (Bobwhite quail, OD 150)
Dietary toxicity to birds	LC_{50} > 5000 mg a.s./kg feed (Bobwhite quail) i.e. > 498 mg a.s./kg bw/d LC_{50} > 6050 mg a.s./kg feed (Mallard duck) i.e. > 475 mg a.s./kg bw/d
Reproductive toxicity to birds	NOEC 802 mg a.s./kg feed (Bobwhite quail) i.e. 74 mg a.s./kg bw/d NOEC 30 mg a.s./kg feed (Mallard duck) i.e. 4.2 mg a.s./kg bw/day

Effects on aquatic organisms

Acute toxicity to fish	LC_{50} 2.54 mg a.s./L (Rainbow trout, tech., static-renewal, 96 h) LC_{50} 2.59 mg a.s./L (Common carp, tech., static-renewal, 96 h) LC_{50} 2.20 mg a.s./L (Bluegill sunfish, tech., static-renewal, 96 h) LC_{50} 2.84 mg a.s./L (Fathead minnow, tech., flow-through, 96 h) LC_{50} > 100 mg p.m./L (Rainbow trout, BYI 08330-enol, static, 96 h) LC_{50} > 100 mg p.m./L (Zebra fish, 4-Methoxycyclohexanone, static, 96 h)
Chronic toxicity to fish	NOEC 0.534 mg a.s./L (Fathead minnow, tech., continuous flow, 33 d)
Bioconcentration	Study not triggered
Acute toxicity to aquatic invertebrates	EC_{50} > 42.7 mg a.s./L (*Daphnia magna*, tech., static, 48 h) EC_{50} > 100 mg p.m./L (*Daphnia magna*, BYI 08330-enol, static, 48 h) EC_{50} > 100 mg p.m./L (*Daphnia magna*, 4-Methoxycyclohexanone, static, 48 h)
Chronic toxicity to aquatic invertebrates	NOEC 2.0 mg a.s./L (*Daphnia magna*, tech., static-renewal, 21 d)
Effect on algal growth	E_rC_{50} 8.15 mg a.s./L (*Pseudokirchneriella subcapitata*, tech., static, 72h) E_rC_{50} 12.17 mg a.s./L (*Navicula pelliculosa*, tech., static, 72h) E_rC_{50} 24.0 mg a.s./L (*Anabaena flos-aquae*, tech., static, 72h) E_rC_{50} > 100 mg p.m./L (*Pseudokirchneriella subcapitata*, BYI 08330-enol, static, 72h)

	E_rC_{50} > 100 mg p.m./L (*Desmodesmus subspicatus*, 4-Methoxycyclohexanone, static, 72h)
Effects on sediment dwelling organisms Acute test	LC_{50} 1.38 mg a.s./L (*Chironomus riparius*, tech., static, 48 h) LC_{50} 74.9 mg p.m./L (*Chironomus riparius*, BYI 08330-enol, static, 48 h) LC_{50} > 100 mg p.m./L (*Chironomus riparius*, BYI 08330-ketohydroxy, static, 48 h) LC_{50} > 100 mg p.m./L (*Chironomus riparius*, BYI 08330-cis-methoxy-cyclohexylamino carboxylic acid, static, 48 h) LC_{50} > 100 mg p.m./L (*Chironomus riparius*, 4-Methoxycyclohexanon, static, 48 h)
Effects on sediment dwelling organisms Chronic test	$EC_{15, ER}$ 0.27 mg a.s./L (*Chironomus riparius*, tech., static, 28 d) $EC_{15, DR}$ > 0.8 mg a.s./L (*Chironomus riparius*, tech., static, 28 d)
Effects on aquatic plants	E_rC_{50} of 6.21 mg a.s./L (*Lemna gibba*, tech., static renewal, 7 d) E_rC_{50} of 19.3 mg p.m./L (*Lemna gibba*, BYI 08330-enol, static, 7 d)

Effects on honeybees

Acute oral toxicity	LD_{50} (48 h): > 107.3 µg a.s./bee LD_{50} (48 h): > 110.7 µg p.m./bee (SPT-cis-enol) LD_{50} (48 h): > 105.5 µg p.m./bee (SPT-cis-ketohydroxy) LD_{50} (48 h): > 109.1 µg p.m./bee (SPT-enol-glucoside) LD_{50} (24 + 48 h): > 101.6 µg p.m./bee (SPT-mono-hydroxy)
Acute contact toxicity	LD_{50} (48 h): > 100 µg a.s./bee LD_{50} (48 h): > 100.0 µg p.m./bee (SPT-cis-enol) LD_{50} (48 h): > 100.0 µg p.m./bee (SPT-cis-ketohydroxy) LD_{50} (48 h): > 100.0 µg p.m./bee (SPT-enol-glucoside) LD_{50} (24 + 48 h): > 100.0 µg p.m./bee (SPT-mono-hydroxy)
Bee brood feeding test	Brood effects were observed in first-tier brood feeding tests at a dietary concentration of 0.0144% a.s.

Effects on other arthropod species

Test Species	Test Substance	Exposure	Application Rate	Ecotoxicological endpoint
		Parasitoids		
Aphidius rhopalosiphi	**OD150**	**Lab, glass plates**	**2; 9; 24; 70 and 200 g a.s./ha**	**LR_{50} 114.7 g a.s./ha** **Reproduction: not assessed**
Aphidius rhopalosiphi	**OD150**	**Ext. lab., barley plants**	**22, 42, 80, 151, 288 g a.s./ha**	**LR_{50} > 288 g a.s./ha** **Reduction of reproduction: -7% at 151 g and 27.8% at 288 g a.s./ha***
		Predatory mites		

Typhlodromus pyri	OD150	Lab., glass cover slides	0.43, 0.19, 0.97; 2.21, 5.0 g a.s./ha	LR_{50} 0.333 g a.s./ha Reproduction: not assessed
Typhlodromus pyri	OD150	Ext. lab., bean leaves	0.15, 0.51, 1.7, 5.9, 20 g a.s./ha	LR_{50} 1.588 g a.s./ha Reduction of reproduction: 43.2% at 0.15 g and 48.7% at 0.51 g a.s./ha
		Foliage-dwelling predators		
Chrysoperla carnea	OD150	Ext. lab., bean leaves	44, 72, 112, 184, 288 g a.s./ha	LR_{50} > 288 g a.s./ha Fecundity reduced by 1.6% in the 184 g and by -0.5% in the 288 g a.s./ha treatment*
Coccinella septempunctata	OD150	Ext. lab., bean leaves	33, 57, 97, 168, 288 g a.s./ha	LR_{50} > 288 g a.s./ha Fertility was reduced by 0.3% at 168 g and by 12.7% at 288 g a.s./ha

*negative values mean increased reproduction/fecundity compared to control

Effects on earthworms

Acute toxicity	LC_{50} (14 d) > 1000 mg a.s./kg d.wt.soil LC_{50} (14 d) > 1000 mg p.m./kg d.wt.soil (BYI 08330-cis-ketohydroxy) LC_{50} (14 d) > 1000 mg p.m./kg d.wt.soil (4-methoxycyclohexanon) LC_{50} > 1000 mg p.m./kg d.wt.soil (BYI 08330-enol)
Reproductive toxicity	NOEC (56 d) = 100 mg p.m./kg d.wt.soil (BYI 08330-enol)

Effects on soil micro-organisms

Nitrogen mineralization	No influence on nitrogen transformation up to 0.96 kg a.s./ha
Carbon mineralization	No influence on carbon transformation up to 0.96 kg a.s./ha

Marine or estuarine organisms

Acute toxicity	LC_{50} 1.96 mg a.s./L (Sheepshead minnow, tech., flow-through, 96 h) EC_{50} 0.85 mg a.s./L (Eastern Oyster, tech., flow-through, 96 h) LC_{50} 5.5 mg a.s./L (Mysids, tech., flow-through, 96 h) E_rC_{50} 1.55 mg a.s./L (Saltwater Diatom, tech., static, 96 h)

Effects on non-target plants

Vegetative vigour	Effects > 50% at a rate of 288 g a.s./ha (non GLP test) EC_{25} 76 g a.s./ha (GLP test)

	EC_{50} > 288 g a.s./ha (GLP test)
Seedling emergence	No significant effects at a rate of 288 g a.s./ha (non GLP test) EC_{25} > 176 g a.s./ha (GLP test)

Effects on other non-target organisms believed to be at risk

Acute and reproductive toxicity	$NOEC_{Reproduction}$ ≥ 1000 mg p.m./kg d.wt.s. (*Hypoaspis aculeifer*, BYI 08330-enol, 14 d)

Effects on biological methods for sewage treatment

Inhibition of respiration rate of the activated sludge	EC_{50} > 10000 mg a.s./L