Document Title

Tier 2 Summary of the Ecotoxicological studies on the Plant Protection Product for Spirotetramat 150 g/L OD

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IIIA1 10 Ecotoxicological studies on the plant protection product

Spirotetramat OD 150 is an oil based suspension concentrate (OD = oil dispersion) containing the active substance spirotetramat at 150 g a.s./L. It is intended to be applied as an insecticide via spray application in lettuce and in citrus crops, with the following application patterns:

Table IIIA1 10-1: Crops and application rates for Spirotetramat OD 150

Crop	Max. single application rate	Max. No. of applications	Max. Σ a.s. [g/ha/season]	Spray interval	Growth stage at application
Citrus	96 g a.s./ha/m CH* max. 3 m height (max. total application rate 288 g a.s./ha)	2	576	21 days	BBCH 71-78
Lettuce	72 g a.s./ha	2	144	14 days	BBCH 42-43

*CH = canopy height

In the following,, the ecotoxicological risk assessment and the study summaries for the product Spirotetramat OD 150 are presented. The Tier 1 assessment will be conducted for citrus and lettuce based on the use pattern presented in Table IIIA1 10-1.

Compound synonyms
The chemical code for spirotetramat is BYI 08330, which is used in some studies, referred to in this dossier.

Metabolites of spirotetramat
Environmentally relevant metabolites of spirotetramat (occurring in soil and/or water with amounts of ≥ 10% of the parent compound) are BYI 08330-enol and BYI 08330-cis-ketohydroxy. These metabolites can occur at relevant concentrations in soil and aquatic ecosystems and are therefore addressed in the risk assessment for soil organisms and for aquatic organisms (for details see section 5, point 9). In addition, the photometabolites BYI 08330-methoxycyclohexanone and BYI 08330-methoxycyclohexylaminocarboxylic acid, which were found with amounts of ≥ 10% of the parent compound in one water photolysis study (Stupp, 2005, KIIA 7.6/02,), are addressed in the aquatic risk assessment. Besides this, plant metabolites of spirotetramat, which were observed in plants metabolism studies at levels above 0.01 mg/kg (BYI 08330–enol, BYI 08330–enol glycoside, BYI 08330-ketohydroxy, BYI 08330-monohydroxy, BYI 08330-di-hydroxy, BYI 08330-desmethyl-enol, and BYI 08330-desmethyl-ketohydroxy (see IIA point 6) were considered in the risk assessment for birds and mammals.

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IIIA1 10.1 Effects on birds

Comment: Detailed descriptions of ecotoxicological studies with birds are given under point 8.1 in the Annex II dossier of spirotetramat.

Birds may be exposed to Spirotetramat OD 150 mainly by the consumption of contaminated feed like insects or via residues on ingested parts of plants. The risk assessment of long-term exposure of birds and the TER_{LT} calculation will be addressed under IIIA1 10.1.2 (short-term toxicity exposure ratio (TER_{ST}) for birds) as there is no individual chapter for long-term exposure of birds intended in the OECD guideline.

Consideration of metabolites:

The following metabolites of spirotetramat were observed in plants metabolism studies at levels above 0.01 mg/kg: BYI 08330–enol, BYI 08330–enol glycoside, BYI 08330-ketohydroxy, BYI 08330-monohydroxy, BYI 08330-di-hydroxy, BYI 08330-desmethyl-enol, and BYI 08330-desmethyl-ketohydroxy .

The enol metabolite is also the primary and main metabolite in the rat and hen metabolism study ([redacted], J., [redacted], A., 2006, KIIA 6.2.2/01). In so far, it can be considered to be ecotoxicologically well characterised and to be covered by the respective bird studies available which were conducted with the parent compound.

BYI 08330-desmethyl-enol was also observed in the rat and hen metabolism study and thus is covered by respective bird studies conducted with parent compound.

Likewise, the ketohydroxy metabolite was detected in rat and hen, and furthermore, an acute rat toxicity study is available for this metabolite (KIIA 5.8/01, [redacted], M., 2005) which suggests that this metabolite shows no toxicity to terrestrial vertebrates (rat $LD_{50} > 5000$ mg a.s./kg bw). This is supported by the observation that the ketohydroxy metabolite proved to be less toxic than the parent compound and than the enol metabolite in all ecotoxicological studies conducted.

The metabolites BYI 08330-monohydroxy, BYI 08330-di-hydroxy and BYI 08330-desmethyl-ketohydroxy showed no toxicity in acute rat studies ($LD_{50} > 5000$ mg a.s./kg bw, see KIIA 5.8/05, [redacted], 2005, KIIA 5.8/07, [redacted], M., 2006 and KIIA 5.8/03, [redacted], M., 2006, respectively) it can thus be considered highly unlikely that exposure might result in unacceptable effects to terrestrial vertebrates.

The enol glycoside metabolite is immediately and quantitatively metabolised to BYI 08330-enol after dietary uptake (see IIA point 6). Thus, this metabolite can be considered to be ecotoxicologically sufficiently covered.

Thereby, the plant metabolites of spirotetramat are covered by existing ecotoxicology or toxicology studies, or they were shown not to be toxic to terrestrial vertebrates. In so far, it is justified to base the risk assessment on spirotetramat for birds on the parent compound.

In case time-weighted average concentrations are considered in the risk assessment, the underlying DT_{50} is based on the measured residue decline of spirotetramat + BYI 08330–enol, since further downstream metabolites have been shown to be non-toxic to terrestrial vertebrates.

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Ecotoxicological endpoints

The following overview table summarises the results of the studies on birds conducted with the active substance spirotetramat. All studies referred to herein have been conducted in compliance with the prevailing OECD or EPA testing guidelines and under GLP.

Table IIIA1 10.1-1: Ecotoxicological endpoints for birds

Test organisms	Duration	Test substance	Reference	Ecotoxicological endpoint
Bobwhite quail	acute	tech.	KIIA 8.1.1/01	LD_{50} > 2000 mg a.s./kg bw
Bobwhite quail	5-day-dietary	tech.	KIIA 8.1.2/01	LDD_{50} > 5000 mg a.s./kg food i.e. > 498 mg a.s./kg bw/d
Mallard duck	5-day-dietary	tech.	KIIA 8.1.3/01	LDD_{50} > 6050 mg a.s./kg food i.e. > 475 mg a.s./kg bw/d
Bobwhite quail	Reproduction	tech.	KIIA 8.1.4/01	NOED 74 mg a.s./kg bw/d
Mallard duck	Reproduction	tech.	KIIA 8.1.4/02	NOAED 9 mg a.s/kg bw/d NOED < 9 mg a.s/kg bw/d
Mallard duck	Reproduction	tech.	KIIA 8.1.4/03	NOED 4.2 mg a.s/kg bw/d

Default Values for Exposure Assessment

The default values for the acute, short-term and long-term exposure in the Tier 1 risk assessment are selected according to recommendations of the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (SANCO/4145/2000 – final). Generic indicator species with specific daily food intake rates (FIR) in different crops are proposed in this guidance document. For spray applications in leafy crops (lettuce) the risk assessment is based on generic data for medium herbivorous and for insectivorous birds. For spray applications in orchards (citrus), insectivorous birds are considered. These generic values are summarised in Table IIIA1 10.1-2.

Table IIIA1 10.1-2: Exposure scenario for spray applications in leafy crops and orchards– default values for acute, short- and long-term exposure. For explanation of the terms referred to, see text below.

Indicator species	Medium herbivorous bird	Insectivorous bird
Crop scenario	Leafy crops	Leafy crops / orchards
Feed	Non-grass herbs	Arthropods
Body weight (bw), indicator species [g]	300	10
FIR: Food (fresh) intake rate [g/d]	228	10.4
FIR related to bw [g *feed*/g *bw*/d]	0.76	1.04
Acute toxicity RUD acute (default value) [(mg *residue*/kg *feed*)/(kg a.s./ha)]	87	52
Short-term / Long-term exposure RUD long-term (default value) [(mg *residue*/kg *feed*)/(kg a.s./ha)]	40	29

- **FIR:** The daily intake of fresh food related to body weight is the quotient of food intake rate (FIR) and body weight.
- **RUD:** residue per unit dose. The RUD is an estimate of typical expected residues on food items [mg a.s./kg] normalised to an application rate of 1 kg a.s./ha. Different percentiles for the residue

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values are used for assessing different scenarios.
For the acute risk assessment, it is assumed that a bird is exposed to food items with residues at the upper end of the residue distribution, i.e. the 90%tile values are used for **RUD acute**.
RUD long-term exposure: for long term exposure scenarios it is very unlikely that one individual will always be exposed to food contaminated with a high level of a plant protection compound. Due to the mobility of the animal, the arithmetic mean value of residues is the more appropriate worst case assumption for this exposure situation.
All RUD data are taken from the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (SANCO/4145/2000-final).

IIIA1 10.1.1 Acute toxicity exposure ratio (TER_A) for birds

The TER figures for the acute exposure of birds are calculated on the basis of the estimated theoretical exposure (**ETE**) related to the daily dietary dose.
According to the provisions of SANCO/4145/2000-final, ETE values in the acute risk assessment for birds are calculated with the formula below. For the acute risk assessment the worst case assumption is made that a bird is exposed to food items with residues at the upper end of the residue distribution, i.e. the 90%tile values have been used.

ETE (acute) = (FIR/bw) × RUD × application rate × MAF × PT × PD

with:

- RUD: RUD acute, see Table IIIA1 10.1-2
- MAF: Multiple Application Factor. In case of repeated applications, the MAF has to be taken into account for calculating the ETE for herbivorous birds. The MAF is a function of the number of applications, interval and DT_{50}. In leafy crops (lettuce) Spirotetramat OD 150 is recommended to be applied at max. 2 times. According to the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC", the MAF for 2 applications is 1.2 (for an application interval of 14 days) in the acute risk assessment. The MAF is not applicable for the estimation of residues in insects.
- PT: Fraction of diet obtained in treated area (number between 0 and 1)
- PD: Fraction of food type in diet (number between 0 and 1; one type or more types) In the Tier 1 worst case approach, PT and PD are set to 100%.

Table IIIA1 10.1.1-1: TER calculation based on acute toxicity and exposure to Spirotetramat OD 150 (use in lettuce). Ecotoxicological endpoint based on bobwhite quail

Application (Spirotetramat OD 150)	Leafy crops (lettuce)	
Max. application rate [kg a.s./ha]	0.072	
Indicator species	Medium herbivorous bird	Insectivorous bird
Feed	Non-grass herbs	Arthropods
FIR/bw [g *feed*/g *bw*/d]	0.76	1.04
RUD [(mg a.s./kg)/(kg a.s./ha)]	87	52
MAF (default)	1.2	Not applicable
ETE [mg a.s./kg/day]	5.7	3.9
LD_{50} [mg a.s./kg/day]	> 2000	
TER_A	**> 351**	**> 513**
Refined Risk Assessment required	**No**	**No**

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Table IIIA1 10.1.1-2: TER calculation based on acute toxicity and exposure to Spirotetramat OD 150 (use in citrus). Ecotoxicological endpoint based on bobwhite quail

Application (Spirotetramat OD 150)	Orchards (citrus)
Max. application rate [kg a.s./ha]	3 x 0.096 = 0.288
Indicator species	Insectivorous bird
Feed	Arthropods
FIR/bw [g *feed*/g *bw*/d]	1.04
RUD [(mg a.s./kg)/(kg a.s./ha)]	52
MAF (default)	Not applicable
ETE [mg a.s./kg/day]	15.6
LD_{50} [mg a.s./kg/day]	> 2000
TER_A	**> 128**
Refined Risk Assessment required	**No**

The acute risk assessment for birds shows that all TER-values are well above the trigger value according to Annex VI, 91/414/EEC ($TER_A \geq 10$) even under the worst case assumptions of a Tier 1 risk assessment (see Tables IIIA1 10.1.1-1 and 10.1.1-2). These results indicate a high margin of safety for birds from the use of Spirotetramat OD 150 under practical conditions. Thus, no unacceptable acute risks to birds are to be expected.

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

The TER figures for the short-term exposure of birds are calculated on the basis of estimated theoretical exposure (ETE) related to the daily dietary dose, as recommended by "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (SANCO/4145/2000-final) according to the following formula:

ETE (short-term) = (FIR/bw) × RUD × application rate × MAF × PT × PD

with:

- RUD: RUD short-term, see Table IIIA1 10.1-2
- MAF: the default value of 1.4 is used for the Tier 1 short-term risk assessment for herbivorous birds, which is recommended for 2 applications and a 14-days interval in lettuce (default value for DT_{50} of 10 days) according to the current version of the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC". The MAF is not applicable for the estimation of residues in insects.
- In the Tier 1 worst case approach, PT and PD are set to 100%.
- Risk assessment is based on the endpoint for the mallard duck, the more sensitive of the species tested

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Table IIIA1 10.1.2-1: TER calculation based on short-term toxicity and exposure to Spirotetramat OD 150 (use in lettuce). Ecotoxicological endpoint based on mallard duck

Application (Spirotetramat OD 150)	Leafy crops (lettuce)	
Max. application rate [kg a.s./ha]	0.072	
Indicator species	Medium herbivorous bird	Insectivorous bird
Feed	Non-grass herbs	Arthropods
FIR/bw [g *feed*/g *bw*/d]	0.76	1.04
RUD [(mg a.s./kg)/(kg a.s./ha)]	40	29
MAF (default)	1.4	Not applicable
ETE [mg a.s./kg/day]	3.1	2.2
LDD_{50} [mg a.s./kg/day]	> 475	
TER_{ST}	**> 153**	**> 216**
Refined Risk Assessment required	**No**	**No**

Table IIIA1 10.1.2-2: TER calculation based on short-term toxicity and exposure to Spirotetramat OD 150 (use in citrus). Ecotoxicological endpoint based on mallard duck

Application (Spirotetramat OD 150)	Orchards (citrus)
Max. application rate [kg a.s./ha]	3 x 0.096 = 0.288
Indicator species	Insectivorous bird
Feed	Arthropods
FIR/bw [g *feed*/g *bw*/d]	1.04
RUD [(mg a.s./kg)/(kg a.s./ha)]	29
MAF (default)	Not applicable
ETE [mg a.s./kg/day]	8.7
LDD_{50} [mg a.s./kg/day]	> 475
TER_{ST}	**> 55**
Refined Risk Assessment required	**No**

The short-term risk assessment for birds shows that all TER-values are well above the trigger value of 10 (according to Annex VI, 91/414/EEC) even under the worst case assumptions of a Tier 1 risk assessment (see Tables IIIA1 10.1.2-1 and 10.1.2-2). These results indicate a high margin of safety for birds from the use of Spirotetramat OD 150 under practical conditions. Thus, no unacceptable short-term risks to birds are to be expected.

Risk Assessment – Long-term Exposure

The TER figures for the long-term exposure of birds are also determined on the basis of estimated theoretical exposure (ETE) related to the daily dietary dose, as recommended by "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (SANCO/4145/2000-final).

According to the SANCO/4145/2000-final, the ETE value for the long-term risk assessment can be obtained with the following formula:

ETE (long-term) = (FIR/bw) × RUD × application rate × MAF × twa-factor× PT × PD

with the following input values at the initial (Tier 1) stage of the risk assessment:

- RUD: RUD long-term, see Table IIIA1 10.1-2
- MAF: The default value of 1.4 is used for the Tier 1 long-term risk assessment for herbivorous birds, which is recommended for 2 applications and a 14-days interval in lettuce (default value for DT_{50} of 10 days) according to the current version of the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC". The MAF is not applicable for the estimation of residues in insects.
- twa-factor: The time-weighted average factor (f_{twa}) accounts for the average concentration of the residues during a certain time interval relative to the initial concentration. The default twa-factor according to the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC", is 0.53, assuming a DT_{50} on herbage of 10 days (= default). The twa-factor is not applicable for residues in insects.
- In the first tier worst-case approach, PT and PD are set to 100%.

Table IIIA1 10.1.2-3: TER calculation based on long-term toxicity and exposure to Spirotetramat OD 150 (use in lettuce). Ecotoxicological endpoint based on bobwhite quail

Application (Spirotetramat OD 150)	Leafy crops (lettuce)	
Max. application rate [kg a.s./ha]	0.072	
Indicator species	Medium herbivorous bird	Insectivorous bird
Feed	Non-grass herbs	Arthropods
FIR/bw [g *feed*/g *bw*/d]	0.76	1.04
RUD [(mg a.s./kg)/(kg a.s./ha)]	40	29
MAF (default)	1.4	Not applicable
twa-factor (default)	0.53	Not applicable
ETE [mg a.s./kg/day]	1.6	2.2
NOED [mg a.s./kg/day]	74	
TER_{LT}	**46**	**34**
Refined Risk Assessment required	**No**	**No**

Table IIIA1 10.1.2-4: TER calculation based on long-term toxicity and exposure to Spirotetramat OD 150 (use in citrus). Ecotoxicological endpoint based on bobwhite quail

Application (Spirotetramat OD 150)	Orchards (citrus)
Max. application rate [kg total a.s./ha]	3 x 0.096 = 0.288
Indicator species	Insectivorous bird
Feed	Arthropods
FIR/bw [g *feed*/g *bw*/d]	1.04
RUD [(mg a.s./kg)/(kg a.s./ha)]	29
MAF (default)	Not applicable
twa-factor (default)	Not applicable
ETE [mg a.s./kg/day]	8.7
NOED [mg a.s./kg/day]	74
TER_{LT}	**8.5**
Refined Risk Assessment required	**No**

Table IIIA1 10.1.2-5: TER calculation based on long-term toxicity and exposure to Spirotetramat OD 150 (use in lettuce). Ecotoxicological endpoint based on mallard duck

Application (Spirotetramat OD 150)	Leafy crops (lettuce)	
Max. application rate [kg a.s./ha]	0.072	
Indicator species	Medium herbivorous bird	Insectivorous bird
Feed	Non-grass herbs	Arthropods
FIR/bw [g *feed*/g *bw*/d]	0.76	1.04
RUD [(mg a.s./kg)/(kg a.s./ha)]	40	29
MAF (default)	1.4	Not applicable
twa-factor (default)	0.53	Not applicable
ETE [mg a.s./kg/day]	1.6	2.2
NOED [mg a.s./kg/day]	4.2	
TER_{LT}	**2.6**	**1.9**
Refined Risk Assessment required	**Yes**	**Yes**

Table IIIA1 10.1.2-6: TER calculation based on long-term toxicity and exposure to Spirotetramat OD 150 (use in citrus). Ecotoxicological endpoint based on mallard duck

Application (Spirotetramat OD 150)	Orchards (citrus)
Max. application rate [kg total a.s./ha]	3 x 0.096 = 0.288
Indicator species	Insectivorous bird
Feed	Arthropods
FIR/bw [g *feed*/g *bw*/d]	1.04
RUD [(mg a.s./kg)/(kg a.s./ha)]	29
MAF (default)	Not applicable
twa-factor (default)	Not applicable
ETE [mg a.s./kg/day]	8.7
NOED [mg a.s./kg/day]	4.2
TER_{LT}	**0.5**
Refined Risk Assessment required	**Yes**

Whereas with the NOED for the quail, the TER is well above the trigger value of 5 (according to Annex VI, 91/414/EEC) even under the worst case assumptions of a Tier 1 risk assessment (see Tables IIIA1 10.1.2-3 and 10.1.2-4), this trigger is not passed with the NOED for the duck. Therefore, a refined risk assessment is presented based on the NOED for the duck endpoint in the following.

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The chronic toxicity data available suggest that the duck is the most sensitive of the species tested. The ecological significance of the reproductive effects found in the chronic duck studies referred to here is unclear at the current status, since they may have been influenced or even caused by atypical foot lesions, which can be significantly aggravated by the artificial conditions of cage maintenance. The chronic risk assessment for birds presented in the following is based on the very conservative lowest endpoint found in a chronic duck study.

Refined chronic risk assessment (mallard duck)

In order to refine the risk assessment based on the duck as most sensitive species, some generic input parameters for the TER calculation according to the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (SANCO/4145/2000-final), and some worst-case assumptions are replaced by more realistic figures, which are more appropriate to reflect field conditions in the respective uses.

These refined input parameters are outlined and justified in detail and summarised below.

Refined chronic risk assessment Lettuce

Species of Concern: Yellow wagtail

The default Tier 1 model species for insectivorous birds (SANCO/4145/2000), the Wren (*Troglodytes troglodytes*) or the Blue Tit (*Parus caeruleus*) cannot be considered particularly relevant for the use of Spirotetramat in lettuce ("leafy crops").

Cramp (1998[1]) described the habitat preference of the Wren to be within a predominantly moist mild climatic range, offering a wide variety of low cover and foraging opportunities, within or outside woodland, crops and aquatic vegetation, fallen trees and branches or heaps of brash, hedgerows, gardens, parks, and shrubberies. In a multi-annual study conducted in the years 1982-84 on the habitat requirements of birds on farmland in south-western Germany ([redacted] 1989, KIIIA1 10.1.2/01, M-115971-01-1), Wrens were recorded 66 times in total per year from which 21 records were made in spring and summer. From these 21 observations recorded during spring and summer, no Wrens were observed foraging in arable fields (average 0%), but most of them foraging in forests (average 63.7%), hedgerows (average 9.7%), trees (average 9.7%) and small forbs (average 5.6%).

Likewise, the Blue Tit, the other insectivorous light weight bird proposed as theoretical worst case model is also of low relevance to vegetable/leafy crops. Rather, the Blue Tit is associated with the presence of trees (Cramp 1998). In the multi-annual study by [redacted] (1989) referred to above, only 0.4% of all Blue Tits were observed in agricultural fields.

Based on its ecology, and the occurrence on arable land, the species listed in Table IIIA1 10.1.2-7 is considered more relevant for the refined risk assessment of spirotetramat in lettuce.

Table IIIA1 10.1.2-7: Relevant insectivorous species of concern for the refined risk assessment of Spirotetramat used in lettuce

Crop	Crop stage	Species group	Example
Lettuce	BBCH 42-43	Insectivorous bird	Yellow wagtail

Yellow Wagtail (*Motacilla flava*): the preference of this species for fields cropped with spring sown vegetables/leafy crops has been reported by various authors:

[1] Cramp S (1998) Birds of the western Palaearctic. CD-ROM version

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1) In a 3-year field study in agricultural land in UK, [redacted] and [redacted] (2000, KIIIA1 10.1.2/02, M-103816-01-1) monitored the territories of eight bird species in eastern England and reported a preference index. The land use in the study area was characterised by winter wheat (32%), winter barley (22%), potatoes (6.4%), oilseed rape (5.2%), salad crops 1.2%), beans (0.8%), grass (8.6%), and set-aside (4.3%). Highest densities of Yellow Wagtails were found in spring crops (potatoes, peas, beans and salad crops). Only a small fraction of the nests monitored (4%) were found in grassland, with a similar proportion in set-aside. Potatoes held the greatest proportion of territories, although nests were found also in spring sown cereals, oil-seed rape, sugar beet, linseed and maize.
2) The typical association of Yellow wagtails with spring-sown leafy row crops was also underlined by [redacted] et al. (2002, KIIIA1 10.1.2/03, M-276031-01-1). The authors monitored territories and nests of Yellow wagtails during their breeding season from May to June 2002 on arable land in Southern Germany. Clearly most territories were counted in spring-sown row crops, (mainly sugar beet and potato but also strawberries) which were significantly preferred over their proportional share.
3) The importance of spring sown row crops for the Yellow wagtail was finally confirmed by a radio-tracking study in potatoes ([redacted] 2005, KIIIA1 10.1.7/02).

This information suggests that the Yellow wagtail is an appropriate focal species for the refined risk assessment for insectivorous birds in lettuce. Although only limited information is directly available from lettuce fields, there are consistent reports of this species in other spring sown leafy row crops. As ecological habitat for insectivorous birds, spring crops like potatoes, sugar beet, and salad crops bear many similarities particularly in late spring / early summer, which is the season relevant to this refined risk assessment addressing the reproductive phase: the leafy crop is grown in rows between birds can walk and forage, with only very limited growth of weeds.
Therefore the refined risk assessment for insectivorous birds in lettuce will be based on the Yellow wagtail as focal species.

Refined chronic risk assessment lettuce: <u>Yellow Wagtail - FIR/bw = 0.88</u>
Yellow wagtails have a body weight of about 17 g ([redacted] & [redacted] 1984, KIIIA1 10.1.2/04, M-001035-01-1). Thus, the average daily food intake can be estimated to amount to 73.7 kJ/day according to Crocker et al. (2002[2]). Arthropods contain on the average 21.9 kJ/g dry weight and consist of 70.5% water (Crocker et al. 2002). Therefore arthropods contain 6.5 kJ/g fresh weight. A yellow wagtail using 73.7 kJ/day will eat 11.4 g arthropods per day. Adjusting this figure for assimilation efficiency (76% for a passerine bird) this results in an average daily food intake of a yellow wagtail of 15 g arthropods per day. Related to the average body weight the FIR/bw will be 0.88.

Refined chronic risk assessment lettuce: <u>Yellow Wagtail - PD = 50% large, ground dwelling + 50% small, foliage dwelling invertebrates</u>
The Yellow Wagtail is primarily an insectivorous bird but also feeds on other epigaeic invertebrates (e.g. spiders). Its foraging behaviour is well known and comprises mainly three techniques: picking (picks items from the ground while walking), run-picking (quick darting run at prey, picking it up either from the ground or as it takes off), and fly-catching (makes short flight from the ground or perch, catching the prey in mid-air). Only occasionally the Yellow Wagtail collects insects from plants in hovering flight (Cramp 1998). Based on these foraging techniques the Yellow wagtail can be expected to feed on a mixed arthropod diet mainly comprising of insects that at least partly dwell on or visit the ground.

[2] Crocker DR, Hart A, Gurney J and McCoy C (2002) Methods for estimating daily food intake of wild birds and mammals. Central Science Laboratory, Project PN0908. Final Report. http://www.pesticides.gov.uk/approvals.asp?id=1183 (from 2006-09-15)

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A targeted study into the prey spectrum of Yellow wagtails on arable land has been conducted by [redacted] (2005). According to his results the Yellow Wagtails foraged on Dipteran, Coleoptera, Aphidoidea, Hymenoptera and Araneae, and targeted specimen larger than the respective average size.
Based on these results it is considered conservative to assume that the typical diet of Yellow Wagtails from lettuce fields consists of 50% large and 50% small insects, taken equally from the foliage and from the ground.

Refined chronic risk assessment lettuce: <u>Yellow Wagtail – Residue per Unit Dose (RUD): 29 (small, foliage dwelling) + 5.1 (large, ground dwelling)</u>

The default RUD for chronic exposure of insectivorous birds preying on small, foliage dwelling invertebrates according to SANCO/4145/2000-final is 29. However, the Yellow Wagtail which has been identified as focal insectivorous species for the refined risk is mainly a ground feeder and forages selectively on larger prey specimen. For large, ground dwelling insects a mean RUD = 5.1 is recommended in SANCO/4145/2000-final.
Since the above mentioned studies showed that the diet of the yellow wagtail is mostly composed of large, ground-dwelling insects, it is a conservative assumption to consider the diet of this bird species to consist of 50% large, ground-dwelling and 50% small, leaf-dwelling insects. The resulting total RUD of the relevant type of diet would herewith be:

$$RUD_{total} = 0.5 \times 5.1\ (RUD_{ground\text{-}dwelling\ insects}) + 0.5 \times 29\ (RUD_{leaf\text{-}dwelling\ insects}) = 17.05$$

Refined chronic risk assessment lettuce: <u>Yellow Wagtail – PT = 0.4</u>

The specific preference of the Yellow Wagtail for spring sown vegetable row crops has been confirmed by various authors. [redacted] & McDonald (2002) reported the highest preference of the Yellow wagtail for potatoes, beans and salad crops, with very similar preference indices of 0.702, 0.693 and 0.752, respectively. [redacted] et al (2002) confirmed the clear preference of Yellow wagtails particularly for potatoes. No data on the specific use of lettuce fields are available but it is reasonable to assume that the use of lettuce field is unlikely to be higher than that of the consistently most attractive crops, i.e. potatoes. As mentioned before, a radio-tracking study has been conducted on the foraging pattern of Yellow Wagtails ([redacted] 2005) in and around potato fields. On average, Yellow Wagtails spent 38.4% of their time in potato fields, 39.8% in cereal fields, 6.8% in oil seed rape, 3.7% on streets and field paths, and 11.3% in other habitats. No data from lettuce fields are available from this study since this crop was not cultivated to a significant extent in the study area, but the results allow nevertheless to expect that Yellow Wagtails will not be exclusively foraging in any specific type of arable fields, including potato fields.
Thus, for the refined risk assessment with the Yellow Wagtail as insectivorous species of concern, the mean portion of diet taken from treated lettuce fields (PT) is considered to be similar to the maximum PT reported from the most preferred crops (potatoes: PT = 0.38). Therefore a PT-value of 0.4 is considered a conservative estimation for the average portion of diet Yellow Wagtails may obtain from lettuce fields treated with Spirotetramat.

Table IIIA1 10.1.2-8: Refined TER calculation based on long-term toxicity and exposure to Spirotetramat OD 150 (use in lettuce). Ecotoxicological endpoint based on mallard duck

Application (Spirotetramat OD 150)	Leafy crops (lettuce)	
Max. application rate [kg a.s./ha]	0.072	
Risk assessment level	Tier 1 (default)	Tier 2 (refined)
Indicator species	Insectivorous bird	Yellow wagtail
Feed	Arthropods	50% large ground dwelling + 50% small foliage dwelling invert.
FIR/bw [g *feed*/g *bw*/d]	1.04	0.88
RUD [(mg a.s./kg)/(kg a.s./ha)]	29	17.05 = (29+5.1)/2
MAF (default)	Not applicable	0.4
twa-factor (default)	Not applicable	Not applied
ETE [mg a.s./kg/day]	2.2	0.43
NOED [mg a.s./kg/day]	4.2	
TER_{LT}	**1.9**	**9.8**
Refined Risk Assessment required	**Yes**	**No**

Refined chronic risk assessment lettuce: Herbivorous bird: <u>Wood pigeon, FIR/bw = 0.76</u>

Wood Pigeon (*Columba palumbus*): unlike the insectivorous bird model at Tier 1, the Wood pigeon can be considered relevant for the refined risk assessment for herbivorous birds in leafy crops. However, direct and quantitative reports about the extent of Wood Pigeons feeding on lettuce are not available. This is not surprising, given the very limited nutritive value of lettuce on one hand, and the protective action vegetable farmers are prepared to take against herbivorous birds ravaging their crops. There are well known examples for Wood Pigeon feeding on vegetable seedlings, particularly on rape and cabbage. In northern France, Wood pigeons are typically considered even as a pest on cabbage.

Refined chronic risk assessment lettuce: <u>Wood Pigeon, PD = 0.2</u>

Overall, Wood Pigeons are herbivorous and feed mainly on grains, fruits, seeds, buds on trees, leaves, root crops and may also take invertebrate food, especially to feed to [redacted] (English Nature [3]). They are known to be particularly fond of the leaves of cabbages and other brassica vegetables.

In a study on the feeding biology of Wood Pigeons in the agricultural landscape, [redacted] et al. (1963, KIIIA1 10.1.2/05, M-251895-01-1) examined the crop contents of shot Wood pigeons. These authors reported a maximum content of 31.8 g kale (cabbage) in the crops of 7 Wood Pigeons (plus 43.2 g clover & 37.3 wheat). Thus, a PD factor of 31.8 / (31.8 + 43.2 + 37.3) = 28.3% could be derived from this study.

However, such PD of 28.3 for cabbage consumption by Wood Pigeon would be an overestimation of exposure to Spirotetramat since in the study by [redacted] et al. (1963), the Wood Pigeons were shot during winter (December – February). In this period, Wood pigeons are forced to forage on leafy crops (particularly winter oil seed rape), since alternative food sources are then at the minimum ([redacted] et al. 1989, KIIIA1 10.1.2/06, M-093498-01-1). No applications of spirotetramat would be made on lettuce in the winter season.

Very similar to the results of [redacted] et al. (1963), [redacted] (1951, KIIIA1 10.1.2/07, M-266530-01-1) reported the share of cabbage to the crop content of shot Wood Pigeons at its maximum of around 25% in January, but with only about 15% in March and no more occurrence until next winter. Thus,

[3] English Nature: Woodpigeon – *Columba palumbus*. http://www.plantpress.com/wildlife/o9-woodpigeon.php

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Wood Pigeons are unlikely to forage preferentially foliage treated with spirotetramat, particularly during the breeding season where plenty and more attractive alternative food is available,

[redacted] et al. (1963) also analysed the feeding habits of Wood Pigeons in the agricultural landscape by analyzing the crop content over the year. During April and May, legume or brassica leaves made up less about 15% and 10% of the diet of 56 and 90 Wood Pigeons, respectively. It is thus considered conservative to use a PD-factor of 0.15 as worst case estimation for the exposure of herbivorous birds to residues in [redacted] leafy crops treated with Spirotetramat.

Despite considerable searching efforts, no single report was detected about wood pigeons feeding on lettuce foliage. This lack of reports suggests that the occurrence of birds foraging on lettuce is probably not wide-spread, and certainly less important than that of birds foraging on cabbage.

The PD-factor of 0.15 as identified for the Wood Pigeon and its wide-spread and well reported foraging preference on cabbages can therefore be considered as appropriate surrogate PD-factor for the use in the exposure assessment of herbivorous birds on lettuce.

Refined chronic risk assessment lettuce: Wood Pigeon, DT_{50} = 3.42 d, MAF = 1.04, 14-d f_{TWA} = 0.33

In the study of [redacted] et al. (2006, KIIIA1 10.1.7/01), the half life of spirotetramat on leaves of soybean, shortgrass, and tallgrass were determined. Mean half-life for soybean was 3.42 days. The half-life on short-grass or tallgrass was even shorter. Considering now soybean as a model plant for the type of diet relevant for herbivorous birds in vegetable crops (non-grass herbs), a MAF of 1.06 and a 14-d twa factor of 0.33 can be derived according to the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (SANCO/4145/2000-final).

Table IIIA1 10.1.2-9: Refined TER calculation based on long-term toxicity and exposure to Spirotetramat OD 150 (use in lettuce). Ecotoxicological endpoint based on mallard duck

Application (Spirotetramat OD 150)	Leafy crops (lettuce)	
Max. application rate [kg a.s./ha]	0.072	
Risk assessment level	Tier 1 (default)	Tier 2 (refined)
Indicator species	Medium herbivorous bird	Wood Pigeon
Feed	Non-grass herbs	Non-grass herbs
FIR/bw [g *feed*/g *bw*/d]	0.76	0.76
RUD [(mg a.s./kg)/(kg a.s./ha)]	40	40
MAF (default)	1.4	1.06
twa-factor (default)	0.53	0.33
PD	1	0.15
ETE [mg a.s./kg/day]	1.6	0.11
NOED [mg a.s./kg/day]	4.2	
TER_{LT}	**2.6**	**38**
Refined Risk Assessment required	**Yes**	**No**

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Refined chronic risk assessment citrus

Spirotetramat OD 150 is intended to be applied in citrus at a maximum single application rate of 96 g a.s./ha/m canopy height in order to control certain scales and other pest organisms. Typically, applications will not be made before end of May / early June. To support the long-term risk assessment for birds, an insect residue study ([redacted] & [redacted], 2008, KIIA 8.16.2/01, M-296043-01-1) has been conducted in Spain. Additionally, [redacted] & [redacted] (2008) present the outline of the results of this study in document M-296043-01-1 (KIIIA1 10.1.2/08). In document M-298940-01-1 (KIIIA1 10.1.2/09) [redacted], [redacted] & [redacted] (2008) present a full refined chronic risk assessment for birds with refined data on the Great tit in citrus orchards, which is considered relevant for the supported application in citrus within this dossier. More details are given below.

Refined chronic risk assessment citrus: <u>measured 21-d TWA residues</u>

In the insect residue study conducted in citrus orchards by [redacted] & [redacted] (2008, KIIA 8.16.2/01, M-296043-01-1), measured 21-d time-weighted averages of 0.35 were determined for ground dwelling arthropods and 1.9 mg/kg for foliage dwelling arthropods.

Refined chronic risk assessment citrus; <u>Species of concern - Great Tit</u>

The default Tier 1 model species for insectivorous birds (SANCO/4145/2000), the Wren (*Troglodytes troglodytes*) or the Blue Tit (*Parus caeruleus*) cannot be considered relevant for the use of Spirotetramat in citrus.

However, published research results underpin the relevance of citrus plantations for Great Tits. Several ecological investigations show that Great Tits find favourable breeding habitats in Spanish citrus groves, that they occur there with relatively high population densities, and that, at least in certain areas of Spain, citrus orchards are even more favourable habitats for Great Tits than the surrounding natural habitats (literature sources are summarised and evaluated in [redacted] & [redacted] 2006 (KIIIA1 10.1.2/10, M-277503-01-1)).

Refined chronic risk assessment citrus: <u>PD for Great tits foraging on foliage dwelling and ground dwelling insects</u>

In Spanish citrus orchards, the diet of Great Tits largely consists of arthropods. A study on the diet composition of great tits foraging in modern citrus orchards in Spain revealed that great tit nestlings in a modern orange grove near Sagunto, East Spain, from April to August 1988 were predominantly fed with Lepidoptera to about 87.8% in numbers of 526 prey items ([redacted] & [redacted] 1990, KIIIA1 10.1.2/11). Among the lepidopterans 49.8% were imagines, almost exclusively Noctuidae, 23.6% were lepidopteran caterpillars, 14.3% lepidopteran pupae ([redacted] & [redacted] 1990). Furthermore the nestlings obtained 5.7% spiders and 6.5% other prey items including Hymenoptera, Coleoptera (Curculionidae), Orthoptera (grass-hoppers), egg cocoons and orange pieces ([redacted] & [redacted] 1990).

Caterpillars were the most abundant prey delivered to the [redacted] only during 6-15 May, while Lepidoptera imagines were more abundant during the rest of the season and thus were considered as an important resource for the great tits breeding in orange groves ([redacted] & [redacted] 1990).

It has become more and more scientifically accepted that - for the residue concentration in prey items - the location of the insect prey is more decisive than its size. Therefore, the results of the insect residue study with Spirotetramat were reported separately for foliage dwelling arthropods (21-d TWA residues: 1.9 mg/kg) and for ground dwelling arthropods (21-d TWA residues 0.35 mg/kg).

Based on feeding observations reported in the literature, an adapted exposure scenario for the great tit feeding in citrus groves on foliage and ground dwelling arthropods is developed.

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Great tits forage mainly in the canopy. However, they also feed on the ground. In mixed woodlands in central Spain, the portion of ground foraging in April and May was reported to be 32% ([redacted] & [redacted] (1997) Fig. 1, KIIIA1 10.1.2/12, M-298655-01-1).
In another study in the UK, great tits foraged during the spring on average for 59% on the ground. As mean value for their entire breeding period from March to August, great tits foraged 31% on the ground ([redacted] 1954, KIIIA1 10.1.2/13, M-289833-01-1).
For the long-term scenario (weeks to months) an average portion of 31% of ground dwelling arthropods may be a realistic estimation of this part of the diet.

Great tit feeding strata	PD
foliar arthropods	0.69
ground arthropods	0.31

Refined chronic risk assessment citrus: PD for the portion of caterpillars in the diet of great tits
Caterpillars can be considered foliage dwelling insects, but - with the conduct of the new insect residue study - it became obvious that caterpillars are no abundant prey any longer in the late spring / early summer season when Spirotetramat will be applied in citrus.
[redacted] & [redacted] (1990) investigated the seasonal variation of the nestling diet of the great tit in orange groves in eastern Spain. The proportion of caterpillars peaked early May and declined to about 10% in the period from May 26th – June 5th (i.e. the expected application timing of Spirotetramat in Spain) and even more in the following weeks (Fig. 1).
This result confirms that caterpillars are not expected to be a significant part of the prey of great tits at the time when Spirotetramat will be applied.
This is consistent with the observation that nearly no caterpillars were sampled in the insect residue study conducted end of May / early June in a Spanish citrus orchard ([redacted] & [redacted] 2007, KIIA 8.16.2/01, M-296043-01-1).
A PD value of 10% for caterpillars will therefore be included in the foliage dwelling arthropod part of the ETE calculation.

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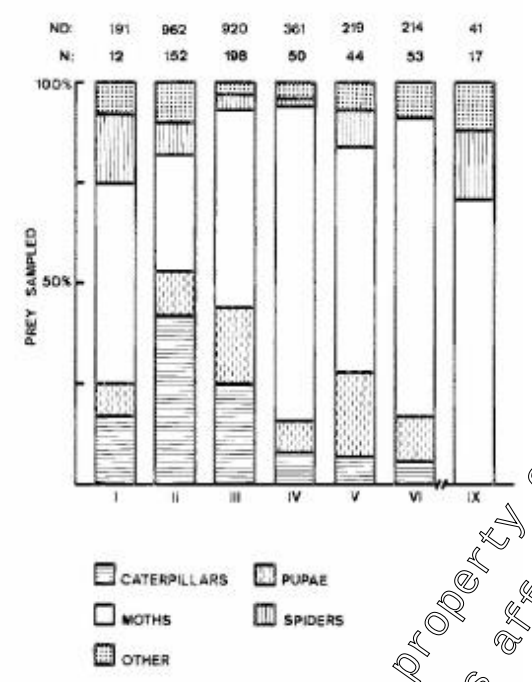

Fig. 1. Seasonal variation in the composition of the diet of nestling Great Tits. Periods are: I (26 April–5 May), II (6–15 May), III (16–25 May), IV (26 May–5 June), V (6–15 June), VI (16–25 June), VII (26 June–5 July), VIII (6–15 July), IX (16–25 July) and X (26 July–5 August). ND is the number of nestling days, and N the number of prey items collected in each period. No samples were collected in periods VII, VIII and X (41, 41 and 4 nestling days, respectively) since all nestlings were either too young or too old for sampling.

Great tit feeding strata	PD
foliar arthropods	0.69 (including 10% caterpillars)
ground arthropods	0.31

Refined chronic risk assessment citrus: PT value for great tits foraging in citrus orchards

Crocker et al [4] (1998, M-041078-01-2, KIIIA1 10.1.2/14) have studied the foraging strategy of insectivorous birds in orchards (results also referred to in SANCO/4145/2000 as possible refinement step). The data indicate that for Blue tits (one of two species default species in the standard scenario) 95% of the local population spent less than 61% of potential foraging time among orchard trees. As the proposed PT value of 0.61 is based on the 95th percentile (instead of the mean PT of 10%) it is very conservative and may also be extrapolated to Great tits in citrus.

Further supportive arguments on the applicability of this PT value in the present risk assessment are supplied in this and the next section.

Research by the Central Science Laboratory in the UK has studied the behaviour of insectivorous birds in orchards (Crocker et al. 1998). The results of this research are cited in the EU Guidance Document SANCO/4145/2000. The data indicate that for blue tits (a common example of a small insectivore, and closely related to the great tit) 95% of the local population spent less than 61% of potential foraging time in the orchard trees. Hence, as a conservative (95th percentile) refinement option, PT may be adjusted to 0.61 for applications in orchards (there is also the potential to use lower percentiles for long

[4] Crocker, D.R., Prosser, P., Tarrant, K.A., Irving P.V., Watola, G., Chandler-Morris, S., Hart J. and Hart A.D.M. (1998). Contract PN0903: Improving the assessment of pesticide risks to birds in Orchards Objective 1: Use of radio-telemetry to monitor birds' use of orchards CSL Report No. EH18/02. http://www.pesticides.gov.uk/approvals.asp?id=1183 (from 2006-09-15)

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term assessments). As this PT value is based on the 95th percentile, it is also proposed to extrapolate to citrus orchards, to provide a general indication of how PT can be refined.
It has been agreed in several circumstances to use the PT value of 0.61 based on Crocker et al. (1998). This refinement approach has been followed for extrapolation from UK to Southern orchard scenarios, as well as for extrapolation from pome to other orchards (i.e. peaches, nectarines, vines) and finally from blue tit to the closely related great tit (in the case of Captan).

In a study conducted by [redacted] (2003, M-266964-01-1, KIIIA1 10.1/14) in the Netherlands on the foraging behaviour of great tits, the birds were attracted to the study orchards by providing nest boxes to compensate for missing nesting sites in modern orchards. This is similar to the situation in the citrus groves studied in Spain ([redacted] & [redacted] 1990). The birds were monitored with binoculars. In the modern apple orchards, an average proportion of foraging trips inside a one-ha orchard of 48% was observed for nine breeding pairs ([redacted], 2003). Therefore, the PT of the great tit in orchards could be set PT = 0.48.

If this is considered a useful support, another new study with data from 25 radio-tracking sessions of 22 great tits in German pome fruit orchards could be submitted on request (mean PT = 0.25; [redacted] & [redacted] 2007, M-291211-01-1, KIIIA1 10.1/15).

All the PT values mentioned above are appropriate for the use in the risk assessment. In the refined TER calculation below, the (most conservative) PT value of 0.61 will be used instead of the lower PT = 0.48 according to [redacted] (2003) or PT = 0.25 according to [redacted] & [redacted] (2007).

Refined chronic risk assessment citrus: <u>Information precluding the use of a PT value < 1 for great tits foraging in citrus orchards</u>

Background: in the original submission the exposure scenario was based on a portion of time (PT) of 61% that great tits spend feeding in the orchard. Justification for the choice of the value is provided in the previous section. This section aims to correct misinterpretations arising from the potentially unclear text in the first submission that may seem to contradict the use of a PT value < 1.
In the original submission, a position paper of [redacted] & [redacted] (2006) was cited which states on page 3 "It was concluded that these orange plantations were a more favourable habitat for the great tit than most natural habitats of this part of Spain ([redacted] et al, 1998, M-290663-01-1, KIIIA1 10.1.2/15)." Taken on its own, this statement on the breeding success of great tits in orange groves in Spain may appear to suggest a high habitat attractiveness and as such question the applicability of PT < 1. However, taking a closer look into the underlying study it becomes clear that there are several additional factors to be considered (e.g, altitude of the locations and time of the observations). Overall, the conclusion of this review will be that citrus groves do not have a particularly high quality as breeding habitat at the time point when spirotetramat will be applied in citrus orchards (end of May / early June and later):
In their study, [redacted] et al (1998) compared the laying date and the size of the first clutch of great tits in a Spanish orange grove with those in other, more natural habitats. Nest boxes were installed in the orange grove and two (sclerophyllous) holm oak forests at different altitudes, a pine forest and a (semi-evergreen) zeen oak forest. Whilst the orange grove was close to the sea level, the forest sites were located at 500, 900-950, 1000-1050 and 900-1100 m altitude.
In the average over 4 years, the mean laying date of the first clutch in the orange plantation (April 21st ± 6.2 days) was about 10-15 days earlier than in the forest habitats.
This time point is significantly earlier than the first applications of Spirotetramat (end of May / early June).
Furthermore, the laying dates were also earlier at low than at high altitude holm oak forest.
The mean size of this first clutch (7.73 ± 0.12 eggs) was slightly higher in the orange plantation than that in three out of the 4 forest habitats (except the "semi-evergreen" zeen oak forest). However, at each

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site the first clutch size was smaller than usually reported from Northern European habitats ([redacted] et al. 1998).

Overall, the earlier laying time and the greater size of the first clutch in the orange grove were linked to the earlier food availability in the evergreen habitats and particularly in the habitats with lower altitude (i.e., the orange grove).

However, an earlier availability of food in the (evergreen) citrus grove cannot be translated into "better food availability later in May and June". In contrary, it is obvious from Fig. 1 in [redacted] & [redacted] (1990) that the typically preferred food of great tits (caterpillars) become rather scarce after the initial peak. Thus, an earlier peak of caterpillars is rather linked to an earlier disappearance (due to various processes including metamorphosis and predation) than indicative of a good caterpillar supply later on. Other research is confirming that early spring advantages are important for tit species. For instance, [redacted] & [redacted] (1989, p. 39, M-298658-01-1, KIIIA1 10.1.2/16) proposed that an "earlier leafing pattern" and a resulting "earlier but also higher food peak" is responsible for an earlier laying date and a larger clutch size of (blue) tits in certain habitats compared to other forest habitats with later prey availability.

In conclusion, the results presented by [redacted] et al. (1998) only concerned the first clutch (produced in April), whilst Spirotetramat will typically be applied not before end of May / early June. Any early season effects which may favour evergreen sea-side level habitats like citrus groves in Spain in the early spring (e.g., early availability of caterpillars) may even have reversed at the Spirotetramat application time, with metamorphosis of the caterpillars, increasing temperature and dryness.

Therefore, the results of [redacted] et al. (1998) do not put into question any proposal for a PT refinement for great tits nesting in citrus groves. The comparison of the breeding habitat quality of the citrus grove (at seaside level) to not-nearby forests (at around 1000 m altitude) in early spring cannot be seen as indicative for the breeding habitat quality of the citrus grove compared to its surroundings in early summer.

Refined chronic risk assessment citrus: Great tit: FIR/bw = 0.85 for arthropods, 1.23 for caterpillars

The food intake rate (FIR) per body weight (bw) of great tits can be calculated to be 0.85 for arthropods (foliage & ground dwelling) and 1.23 for caterpillars (foliage dwelling).

Table IIIA1 10.1.2-10: Food intake rate (FIR) in great tits

Feed item	Energy content 1)	Moisture content 1)	Energy content 1)	Daily energy demand 2)	Assimilation efficiency 1)	FIR	FIR/bw
	[kJ/g dry weight]	[%]	[kJ/g fresh weight]	[g fresh weight/day]	[%]	[g fresh weight/day]	[g fresh weight/kg bw/day]
Arthropods	21.9	70.5	6.5	79.6	76	16.2	**0.85**
Caterpillars	21.7	79.4	4.5	79.6	76	23.4	**1.23**

1) Data derived from Crocker et al. (2002) [5]
2) Based on a body weight of 19.0 g (Dunning, 1993)[6]; and if exclusively feeding on the respective feed item

[5] Crocker, D. R., Hart, A. D. M., Gurney, J. & McCoy, C. 2002. Project PN0908: Methods for estimating daily food intake of wild birds and mammals. pp. 1-22. York: Central Science Laboratory.
[6] Dunning, J. B. 1993. CRC handbook of avian body masses. Boca Raton, Ann Arbor, London, Tokyo: CRC Press.
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Refined chronic risk assessment citrus: Great tit: ETE calculation

As outlined above, the daily ETE can be calculated to be 0.78 mg/kg bw/d with

- FIR/bw values of 0.85 for arthropods and 1.23 for caterpillars,
- the measured 21-d time-weighted averages from the insect residue study (0.35 and 1.9 mg/kg for ground and foliage dwelling arthropods),[7]
- the PD values of 31% ground dwelling and 69% foliage dwelling (including 10% caterpillars),
- and the PT value of 0.61

Table IIIA1 10.1.2-11: ETE calculation

Food item	bw	FIR/bw	residues 21-d twa	PD	PT	ETE
Foliar arthropods	19	0.85	1.9	0.59	0.61	0.58
Caterpillars (foliar)	19	1.23	1.9	0.10	0.61	0.14
Ground arthropods	19	0.85	0.35	0.31	0.61	0.06
					Total ETE	**0.78**

Table IIIA1 10.1.2-12: Refined TER calculation based on long-term toxicity and exposure to Spirotetramat OD 150 (use in citrus). Ecotoxicological endpoint based on mallard duck

Application (Spirotetramat OD 150)	Orchards (citrus)	
Max. application rate [kg total a.s./ha]	3 x 0.096 = 0.288	
Risk assessment level	Tier 1 (default)	Tier 2 (refined)
Indicator species	Insectivorous bird	Great tit
Feed	Arthropods	Mixed diet see Table IIIA1 10.1.2-11
FIR/bw [g *feed*/g *bw*/d]	1.04	0.85 for foliar arthropods 1.23 for caterpillars (foliar) 0.85 for ground arthropods
RUD [(mg a.s./kg)/(kg a.s./ha)]	29	-
MAF (default)	Not applicable	-
twa-factor (default)	Not applicable	-
Residues 21-d twa [(mg a.s./kg)/(kg a.s./ha)]	-	1.9 for foliar arthropods 1.9 for caterpillars (foliar) 0.35 for ground arthropods
PD	1	0.59 for foliar arthropods 0.10 for caterpillars (foliar) 0.31 for ground arthropods
PT	1	0.61
ETE [mg a.s./kg/day]	8.7	0.78 (Table IIIA1 10.1.2-11)
NOED [mg a.s./kg/day]	4.2	
TER_{LT}	**0.5**	**5.4**
Refined Risk Assessment required	**Yes**	**No**

Compared to the lowest NOEL of 4.2 mg/kg bw/d obtained in a mallard duck study, the refined TER amounts to 5.4.

Using one of the other proposed PT values (0.48 or 0.25), and/or with the geometric mean NOEL for reproductive effects on mallard duck and bobwhite quail, even higher TER values would be achieved.

[7] Only a single application is considered here, since a second application would be late in the year and without significance for the breeding period of the birds

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

Not relevant as the product will be used as spray application.

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

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

Not relevant as the product will be used as spray application.

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

Not relevant as the product will be used as spray application.

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

Not relevant as the product will be used as spray application.

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

According to the Guidance Document on Terrestrial Ecotoxicology, studies with the formulation are considered necessary only where they will clearly add essential information which is more typically the case for specific, e.g. granular formulations. Since Spirotetramat OD 150 is a spray formulation with only one active substance which was found virtually void of any acute toxicity in birds, a further acute study on birds with the formulation was not taken into consideration due to animal welfare reasons. For results of the acute toxicity of the active substance refer to the corresponding Annex II 8.1.1.

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IIIA1 10.1.7 Supervised cage or field trials

Report:	**KIIIA1 10.1.7/01, [redacted], [redacted], T. & [redacted], L.; 2006**
Title:	BYI08330 150 OD - Magnitude of the Residue in/on Soybeans/Wheat, Potential Wildlife Feed Items. Date: 2006-09-06
Organisation:	Bayer CropScience, Ecotoxicology, [redacted], Kansas
Report No.:	RAFNP002; M-277315-01-1
Publication:	unpublished
Dates of experimental work:	June 03, 2005 – June 23, 2006
Guidelines:	Not applicable
Deviations:	Not applicable
GLP	Yes (certified laboratory)

Material and methods

Test item: Spirotetramat (BYI 08330) OD 150, 150 g a.s./L, nominal, specified by batch no. 08030/0189(0152)

Four soybean field trials and four wheat field trials were conducted in NAFTA Growing Regions 2 (one soybean trial), 3 (one wheat trial), 4 (one soybean trial and one wheat trial), and 5 (two soybean trials and two wheat trials). At each test site, two foliar applications of BYI 08330 150 OD were made to established soybeans or wheat at a target application rate of 0.157 lb a.s./A/application (0.176 kg/ha/application) and with an application interval of 19 to 21 days. The wheat trials included two treated plots; one plot was mowed one to eight days prior to the applications and represented short grass; the other plot was not mowed and represented tall grass. Duplicate forage samples were collected from each of the treated plots at each of the sampling intervals from the soybean and wheat trials. The forage samples were collected prior to the first application (control samples) and at various time intervals after the first and second applications, up to 35 days following the second application. The samples were homogenized, and the residues of BYI 08330 and its metabolites BYI 08330-enol, BYI 08330-ketohydroxy, BYI 08330-mono-hydroxy, and BYI 08330-enol-glucoside were quantified by high pressure liquid chromatography/triple stage quadrupole mass spectrometry (lc-ms/ms) using the stable isotopically labelled analytes as internal standards and the method of external standard quantitation. The individual analyte residues were converted to BYI 08330 molar equivalents and summed to give a total BYI 08330 residue. The foliar dissipation half-life of the total BYI 08330 residue in soybean forage (broad leaf plants) and wheat forage (short and tall grass) following the second application of BYI 08330 150 OD was calculated.

Findings

Residues in Soybean Forage (Broad Leaf Plants) and Wheat Forage (Short and Tall Grass) Following the Second Application of BYI 08330 150 OD and Calculated Half-lives

Crop	Mean Total BYI 08330 Residue Immediately Following the Second Application [ppm][a]	Maximum Total BYI 08330 Residue Following the Second Application [ppm][b]	Mean Half-Life [days][c]
Soybean Forage	8.32	10.28	3.42[d]
Wheat Forage (Short Grass)	14.35	20.35	2.13
Wheat Forage (Tall Grass)	13.04	21.65	1.96

[a] Mean Total BYI 08330 Residue Immediately Following the Second Application is the average from all of the trials of the total BYI 08330 residue from the sample interval immediately following the second application of BYI 08330 150 OD.

[b] Maximum Total BYI 08330 Residue Following the Second Application is the maximum total BYI

08330 residue from all of the trials for the sample intervals following the second application of BYI 08330 150 OD.

[c] Mean Half-life is the average of the half-lives calculated for each trial from the total BYI 08330 residue at each of the sample intervals following the second application of BYI 08330 150OD.

[d] The Mean Half-life for total BYI 08330 residue in soybean forage is 4.45 days without inclusion of the half-life from trial FN333-05W which received 1.3 inches of rain 1 day following the second application of BYI 08330 150 OD.

The highest peak residue level detected was in unmown wheat (21.65 ppm) which was slightly higher as compared to mown wheat (20.35 ppm) and soybean foliage (10.28 ppm).

Observations

Precipitation totals between the time of the first application and the final harvest date ranged from 2.58 to 6.07 inches. Rainfall did not occur on the day of application for any of the trials. For any of the trials, the earliest that rainfall occurred following application of BYI 08330 150 OD was in trials FN331-05W and FN333-05W which received rain (0.02 inches and 1.3 inches, respectively) 1 day following the second application.

Conclusions

Total BYI 08330 residue dissipated rapidly in soybean forage (broad leaf plants) and wheat forage (short and tall grass) after application of BYI 08330 150 OD at the highest proposed seasonal use rate.

Report:	**KIIIA1 10.1.7/02, [redacted], Ch., 2005**
Title:	Generic Field Monitoring of Birds in Potato Cultivation in Northern Germany.
	Date: 2004-09-29, Amended: 2005-01-04
Organisation:	Bayer CropScience AG, [redacted], Germany
Report No.:	WFC/FS 019; M-090336-02-1
Publication:	unpublished
Dates of experimental work:	2004-05-28 to 2004-09-17
Guidelines:	The test was especially designed for the purpose of this study.
Deviations:	not applicable
GLP/GEP	Yes

Material and methods

The study has been conducted in and around six different potato fields in the "[redacted]" region near the village [redacted] between the towns of [redacted] and [redacted], in Lower Saxony, Germany. This region is a typical area of potato cultivation in Europe and known to hold a population of Yellow Wagtails.

To identify bird species of concern for potato fields (besides the Yellow Wagtail) and to appraise the relevancy of potato fields and other habitats as feeding habitats for birds, census counts were carried out along different transects, representing all main agrarian habitats within the study area.

20 Yellow Wagtails were trapped on and in the margin of potato fields, tagged with radio transmitters and tracked for one to five daylight periods each. The location, habitat and behaviour was recorded continuously to get information of the home range, habitat selection and time budget of birds using potato fields living in a cultivable area for potatoes.

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To get information about the food items, actually selected by the Yellow Wagtails, samples of faeces were analysed quantitatively for composition (taxonomic orders of arthropods, other items).

Findings:

Results of monitoring

PORTION OF TIME (PT) in habitat of Yellow Wagtails after radio tracking			
potential foraging time (sum of behaviour categories “foraging”+“active”+“unknown”) spent per habitat; based on 20 individuals, 1 to 5 24-hour sessions each [mean of individuals], (90%ile)	potato fields	38.4 %	(72.3)
	cereal fields	39.8 %	(44.1)
	oil seed rape fields	6.8 %	(22.2)
	street and field path	3.7 %	(10.7)
	other habitats	11.3 %	(16.0)

FEEDING HABITAT of Yellow Wagtails after transect counts (based on population)		
abundance of potentially foraging individuals of the population after 9 transect counts covering 48.58 ha field crops each [individuals/ha]	potato fields	0.60
	cereal fields	0.41
	sugar beet fields	0.32
	oil seed rape fields	0.22

PREFERENCE OF POTATO FIELDS in Yellow Wagtails after radio tracking	
preference of potato fields as a feeding habitat [Jacobs’ index (D), MCP (100%)]	0.16

DIET of Yellow Wagtails			
main arthropod orders actually eaten by Wagtails foraging in and around potato fields (based on 25 samples of faeces)	**taxonomic order**	**steadiness**[1]	**mean number**[2]
	Diptera	100%	3.96
	Coleoptera	84%	3.60
	Aphidoidea	72%	5.28
	Hymenoptera	52%	0.80
	Aranea	48%	0.64

SIZE OF ARTHROPODS actually chosen by Yellow Wagtails			
Mean sizes of actually eaten individuals (after samples of faeces) compared to mean sizes of unselective samples of the population in potato fields	**taxonomic order**	**food** [mm] (n)	**population** [mm] (n, stratum[3])
	Diptera	7.49 (99)	2.78 (523, f)
	Coleoptera	7.22 (90)	5.85 (128, g)
	Aphidoidea	3.00 (132)	1.98 (350, f)
	Hymenoptera	6.33 (20)	3.13 (25, f)
	Aranea	4.38 (16)	3.12 (58, g)

MAIN BIRD SPECIES in potato fields potentially foraging		
mean abundance of main species after 9 transect counts covering 9.98 ha potato fields each	Yellow Wagtail	0.60 individuals per ha
	Pied Wagtail	0.29 individuals per ha
	Whitethroat	0.12 individuals per ha

[1] portion of samples containing this type
[2] mean number of individuals per sample
[3] f = foliage dwelling arthropods, obtained by inventory spraying; g = ground dwelling arthropods, obtained by pitfall trapping

Conclusion

Radio-tracking of 20 individual Yellow Wagtails (each for a minimum of 24 and a maximum of 120 hours) in an agrarian landscape with a high number of potato fields in the north-western part of Lower Saxony showed that this field type was used as a main feeding habitat by Yellow Wagtails. However, cereal fields (barley, wheat and rye) have been used as well to a (in summary) similar high proportion

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by Yellow Wagtails, which foraged for chick provisioning or the bird's own use. Only one individual fed almost exclusively on potato fields while tracking, while one individual did not use potato fields as feeding habitat at all.
Potato fields were on average selected to a slightly higher proportion for foraging as to be derived from the available proportion in their home range [Jacobs' index (D)]. Thus Yellow Wagtails positively selected potatoes as a foraging habitat, but only to a little extent.
Tracking data of individual Yellow Wagtails were confirmed by census data of the population of this species within the study area. Moreover census data confirmed the Yellow Wagtail to be the most relevant bird species in potato.
To sum up, it can be ascertained, that potato fields offered a significant but not exclusive feeding habitat for the tracked Yellow Wagtails.

For risk assessment purposes a value for portion of time spent foraging in potato fields (PT) can be derived for Yellow Wagtails from the study results: Yellow Wagtails settling in or in close vicinity to potato fields have been on average 38.4% of their potentially foraging time in potato fields (90th percentile 72.3%).

Food of Yellow Wagtails in and around potato fields was dominated by Diptera, Coleoptera and Aphidoidea. In all taxonomic arthropod orders large individuals were selected distinctly.

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

Not relevant as the product will be used as spray application.

IIIA1 10.1.9 Effects of secondary poisoning

Crop protection products with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds if contaminated prey like fish or earthworms are taken up.
For spirotetramat, the low log P_{OW} of 2.51 (at pH 7, see KIIA 2.8.1; Lemke, Mühlenberg, 2003) indicates that a significant accumulation in potential prey organisms has not to be expected. Thus, based on the low log P_{OW} of the active substance, a risk assessment to account for secondary poisoning is not considered necessary.
This applies also to the metabolites of spirotetramat as the log P_{OW} of BYI 08330-cis-ketohydroxy is 1.3 (KIIA 7.13/05; Bogdoll, Lemke, 2006) and of BYI 08330-enol is 0.3 (at pH 7, see KIIA 7.13/02; Eyric, Bogdoll, 2006). For the metabolites BYI 08330-Methoxycyclohexanone and BYI 08330-Methoxycyclohexylaminocarboxylic acid log P_{OW} values of 0.29 and - 2.02 were estimated with EPA program KOWWIN v1.67.

IIIA1 10.2 Effect on aquatic organisms

Ecotoxicological endpoints

Table IIIA1 10.2-1: Ecotoxicological endpoints for aquatic organisms exposed to the active substance spirotetramat

Test organisms	Test system	Test substance	Reference	Ecotoxicological endpoint
Fish, acute				
Rainbow trout	static-renewal, 96 h	tech.	KIIA 8.2.1.1/01	LC_{50} 2.54 mg a.s./L[1]
Common carp	static-renewal, 96 h	tech	KIIA 8.2.1.2/01	LC_{50} 2.59 mg a.s./L[1]
Bluegill sunfish	static-renewal, 96 h	tech.	KIIA 8.2.1.2/02	LC_{50} 2.20 mg a.s./L
Fish, chronic				
Fathead minnow	continuous flow, 33 d	tech.	KIIA 8.2.4/01	NOEC 0.534 mg a.s./L[1]
Freshwater invertebrates, acute				
Daphnia magna	static, 48 h	tech.	KIIA 8.3.1.1/01	EC_{50} > 42.7 mg a.s./L[1]
Freshwater invertebrates, chronic				
Daphnia magna	static-renewal, 21 d	tech.	KIIA 8.3.2.1/01	NOEC 2.0 mg a.s./L
Sediment-dwelling organisms, acute				
Chironomus riparius	static, 48 h, water-only	tech.	KIIA 8.5.1/01	LC_{50} 1.38 mg a.s./L[2]
Sediment-dwelling organisms, chronic				
Chironomus riparius	static, 28 d, spiked water	tech.	KIIA 8.5.2/01	$EC_{15\ ER}$ 0.27 mg a.s./L[2] $EC_{15\ DR}$ > 0.80 mg a.s./L[2]
Algae				
Anabaena flos-aquae	static, 96 h	tech.	KIIA 8.4/03	E_rC_{50} (72 h) 24.0 mg a.s./L[3]
Navicula pelliculosa	static, 96 h	tech.	KIIA 8.4/02	E_rC_{50} (72 h) 12.17 mg a.s./L[3]
Pseudokirchne-riella subcapitata	static, 72 h	tech.	KIIA 8.4/01	E_rC_{50} 8.15 mg a.s./L[1]
Aquatic plants [4]				
Lemna gibba	7 d, static-renewal	tech.	KIIA 8.6/01	E_rC_{50} 6.21 mg a.s./L[1]

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Table IIIA1 10.2-1: continued

Test organisms	Test system	Test substance	Reference	Ecotoxicological endpoint
Marine organisms				
Sheepshead minnow	flow-through, 96 h	tech.	KIIA 8.11.1/01	LC_{50} 1.96 mg a.s./L
Eastern oyster	flow-through, 96 h	tech.	KIIA 8.11.1/02	EC_{50} 0.85 mg a.s./L
Mysid shrimp	flow-through, 96 h	tech.	KIIA 8.11.1/03	EC_{50} 5.5 mg a.s./L
Skeletonema costatum	static, 96 h	tech.	KIIA 8.11.1/04	E_rC_{50} (72h) 1.55 mg a.s./L

[1] based on mean measured concentrations
[2] based on nominal initial concentrations
[3] based on initial measured concentrations
[4] The risk assessment for aquatic plants is presented in chapter IIIA1 10.8.2.

Table IIIA1 10.2-2 Ecotoxicological endpoints for aquatic organisms exposed to Spirotetramat OD 150

Test organisms	Test system	Test substance	Reference	Ecotoxicological endpoint
Fish, acute				
Rainbow trout	static, 96 h	OD 150	KIIIA1 10.2.2.1/01	LC_{50} 1.43 mg a.s./L
Sediment-dwelling organisms, acute				
Chironomus riparius	static, 48 h, water-only	OD 150	KIIIA1 10.2.2/01	LC_{50} 0.82 mg a.s./L
Algae				
Pseudokirchneriella subcapitata	static, 72 h	OD 150	KIIIA1 10.2.2.3/01	E_rC_{50} > 8.2 mg a.s./L

Remark: Since *Daphnia* was shown to be less sensitive to the active compound than the most sensitive freshwater species (*Chironomus*) by a factor greater than 30 in terms of acute toxicity, an acute toxicity study on *Daphnia* with the OD 150 formulation was deemed not necessary. The risk for *Daphnia* is considered to be covered by the risk assessment for the most sensitive species *Chironomus*.

Consideration of metabolites:

Since BYI 08330-enol and BYI 08330-cis-ketohydroxy were found in concentrations of ≥ 10% of the parent compounds in aquatic systems, they are considered in the aquatic risk assessment.

For BYI 08330-enol tests were conducted with fish, *Daphnia*, algae, aquatic plants and chironomids. The metabolite BYI 08330-cis-ketohydroxy, however, which is formed by degradation of the enol metabolite, was only tested with *Chironomus riparius* as this species was shown to be the most sensitive taxon in tests with the parent compound (see Table IIIA1 10.2-1). Although *Lemna* was shown to be slightly more sensitive to the enol metabolite than *Chironomus*, sensitivities of both organisms are in the same order of magnitude. Furthermore, as it is indicated by TER values greater than 1000 (even for *Lemna* as high as 1206), the enol metabolite is virtually non-toxic to aquatic organisms (see below). As the endpoint for the ketohydroxy metabolite in the test on *Chironomus* suggests, this metabolite is even less toxic to aquatic organisms than the enol metabolite. Therefore, even if *Lemna* would be slightly more sensitive to BYI 08330-cis-ketohydroxy than *Chironomus*, the risk assessment based on *Chironomus* would still be fully protective for *Lemna* as well.

As the *Chironomus* LC_{50} of > 100 mg p.m./L reveals a toxicity which is more than 100 times lower compared to the parent compound, no further studies on aquatic organisms with BYI 08330-cis-

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ketohydroxy were deemed necessary and the risk is considered to be covered by the risk assessment for the parent compound.
The photometabolites BYI 08330-methoxycyclohexanone and BYI 08330-methoxy cyclohexylamino-carboxylic acid were found with amounts of ≥ 10% of the parent compound in one water photolysis study (Stupp, 2005KIIA 7.6/02). For both metabolites studies on the most sensitive aquatic organism (*Chironomus*) resulted in LC_{50} of >100 mg metabolite/L. In addition, toxicity data for fish, Daphnia and algae are available for BYI 08330-methoxycyclohexanone.

Table IIIA1 10.2-3: Ecotoxicological endpoints for aquatic organisms (metabolites of spirotetramat)

Test organisms	Test system	Test substance	Reference	Ecotoxicological endpoint	
BYI 08330-enol					
Rainbow trout	static, 96 h	metabolite	KIIA 8.2.1.3/01	LC_{50}	> 100 mg p.m./L
Daphnia magna	static, 48 h	metabolite	KIIA 8.3.1.1/02	EC_{50}	> 100 mg p.m./L
Chironomus riparius	static, 48 h, water-only	metabolite	KIIA 8.5.1/02	LC_{50}	74.9 mg p.m./L[1]
Pseudokirchneriella subcapitata	static, 72 h	metabolite	KIIA 8.4/04	E_rC_{50}	> 100 mg p.m./L
Lemna gibba[2]	7 d, static	metabolite	KIIA 8.6/02	E_rC_{50}	19.3 mg p.m./L[1]
BYI 08330-ketohydroxy					
Chironomus riparius	static, 48 h, water-only	metabolite	KIIA 8.5.1/03	LC_{50}	> 100 mg p.m./L
BYI 08330-methoxy cyclohexylamino carboxylic acid					
Chironomus riparius	static, 48 h, water-only	metabolite	KIIA1 8.5.1/04	LC_{50}	> 100 mg p.m./L
BYI 08330-methoxy cyclohexanone*					
Zebra fish	static, 96 h	metabolite	KIIA 8.2.1.3/02	LC_{50}	> 100 mg p.m./L
Daphnia magna	static, 48 h	metabolite	KIIA 8.3.1.1/03	EC_{50}	> 100 mg p.m./L
Chironomus riparius	static, 48 h, water-only	metabolite	KIIA1 8.5.1/05	LC_{50}	> 100 mg p.m./L
Desmodesmus subspicatus	static, 72 h	metabolite	KIIA 8.4/05	E_rC_{50}	> 100 mg p.m./L

p.m. = pure metabolite
[1] based on nominal initial concentrations
[2] the risk assessment for *Lemna* is presented in chapter IIIA1 10.8.2.
* = 4-Methoxycyclohexanone

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IIIA1 10.2.1 Toxicity exposure ratios for aquatic species

Aquatic organisms may be exposed to some extent by spray drift, dry deposition, run-off and drainage from treated fields. The predicted environmental concentrations in surface water (PEC_{SW}) of spirotetramat (BYI 08330) and its main aquatic metabolites, BYI 08330-enol, BYI 08330- ketohydroxy, BYI 08330-methoxycyclohexanone and BYI 08330-methoxycyclohexylaminocarboxylic acid, were calculated according to FOCUS by [redacted] (2006, report MEF-06/281 for citrus and MEF-06/386 for lettuce) for the use of the insecticide by spray application in citrus and leafy vegetables (i.e. lettuce). FOCUS STEP 2 calculations were conducted for the parent compound and all environmentally relevant metabolites. FOCUS STEP 3 and 4 calculations were conducted for the parent compound only. The calculated maximum PEC_{SW} values for spirotetramat and its metabolites according to FOCUS STEP 2 are summarised in Table IIIA1 10.2.1-1.

Table IIIA1 10.2.1-1: Max. PECsw values for spirotetramat and its metabolites according to FOCUS STEP 2. Values in bold italics are used for the risk assessment as worst case assumptions.

Crop	Spirotetramat [µg a.s./L]	BYI 08330-enol [µg p.m./L]	BYI 08330-ketohydroxy [µg p.m./L]	4-Methoxy-cyclo-hexanone [µg p.m./L]	BYI 08330-methoxy cyclohexylamino carboxylic acid [µg p.m./L]
Citrus	***11.643***	***15.583***	***8.586***	***1.387***	***1.206***
Lettuce	0.585	0.950	0.662	0.070	0.061

The values in ***bold italics*** represent the worst case, covering all other application scenarios. These values are used for the Tier 1 TER calculation.

IIIA1 10.2.1.1 TER_A for fish

The following acute TER calculations are based on the endpoints for fish exposed to spirotetramat and its metabolites.

Table IIIA1 10.2.1.1-1: Acute TER for fish exposed to spirotetramat and its metabolite

Test Organism, time scale	Test substance	Ecotoxicological endpoint	PEC_{max} [mg/L]	TER	Refine-ment required?
Spirotetramat					
Rainbow trout static-renewal, 96 h	a.s.	LC_{50} 2.54 mg a.s./L	0.0116	**219**	**No**
Rainbow trout 96 h, static	OD 150	LC_{50} 1.43 mg a.s./L		**123**	**No**
Common carp static-renewal, 96 h	a.s.	LC_{50} 2.59 mg a.s./L		**223**	**No**
Bluegill sunfish static-renewal, 96 h	a.s.	LC_{50} 2.20 mg a.s./L		**190**	**No**
BYI 08330-enol					
Rainbow trout, static, 96 h	metabolite	LC_{50} > 100 mg p.m./L	0.0156	**> 6,410**	**No**
4-Methoxycyclohexanone					
Zebra fish, static, 96 h	metabolite	LC_{50} > 100 mg p.m./L	0.0014	**>71,429**	**No**

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As shown in Table IIIA1 10.2.1.1-1, the TER-values for the formulated product Spirotetramat OD 150, spirotetramat and its metabolites BYI 08330-enol and 4-Methoxycyclohexanone exceed the trigger value of 100, indicating no need for a refined risk assessment considering acute toxicity to fish.

IIIA1 10.2.1.2 TER_{LT} for fish

The following long-term TER calculation is based on the endpoint for fish exposed to spirotetramat.

Table IIIA1 10.2.1.2-1: Long-term TER for fish exposed to spirotetramat

Test Organism, time scale	Test substance	Ecotoxicological endpoint	PEC_{max} [mg/L]	TER	Refine-ment required?
Fathead minnow continuous flow, 33 d	a.s.	NOEC 0.534 mg a.s./L	0.0116	**46**	**No**

As shown in Table IIIA1 10.2.1.2-1, the TER-value for spirotetramat is well above the trigger value of 10, indicating no need for a refined risk assessment considering long-term toxicity to fish.

IIIA1 10.2.1.3 TER_A for Daphnia

The following acute TER calculations are based on the endpoints for *Daphnia* exposed to spirotetramat and its metabolites. Since *Daphnia* is the aquatic organism least sensitive to spirotetramat (less sensitive than *Chironomus riparius*, the most sensitive species, by a factor greater than 30), a formulation study was not conducted with this species. The risk assessment for *Daphnia* is therefore based on the endpoint of the a.s. study.

Table IIIA1 10.2.1.3-1: Acute TER for *Daphnia* exposed to spirotetramat and its metabolites

Test Organism, time scale	Test substance	Ecotoxicological endpoint	PEC_{max} [mg/L]	TER	Refine-ment required?
Spirotetramat					
Daphnia magna static, 48 h	a.s.	EC_{50} > 42.7 mg a.s./L	0.0116	**> 3681**	**No**
BYI 08330-enol					
Daphnia magna static, 48 h	metabolite	EC_{50} > 100 mg p.m./L	0.0156	**> 6410**	**No**
BYI 08330-methoxycyclohexanone					
Daphnia magna static, 48 h	metabolite	EC_{50} > 100 mg p.m./L	0.0014	**>71,429**	**No**

As shown in Table IIIA1 10.2.1.3-1, the TER-values for spirotetramat and its metabolites BYI 08330-enol and BYI 08330-methoxycyclohexanone exceed by far the trigger value of 100, indicating no need for a refined risk assessment considering acute toxicity to *Daphnia magna*.

IIIA1 10.2.1.4 TER_{LT} for Daphnia

The following long-term TER calculation is based on the endpoint for *Daphnia* exposed to spirotetramat.

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Table IIIA1 10.2.1.4-1:Long-term TER for *Daphnia magna* exposed to spirotetramat

Test Organism, time scale	Test substance	Ecotoxicological endpoint	PEC_{max} [mg/L]	TER	Refine-ment required?
Daphnia magna static-renewal, 21 d	a.s.	NOEC 2.0 mg a.s./L	0.0116	**172**	**No**

The TER value of spirotetramat is well above the Annex VI trigger value of ≥ 10. Thus, a refinement of the chronic *Daphnia* risk assessment is not required.

IIIA1 10.2.1.5 TER_A for an aquatic insect species

The acute TER for an aquatic insect species is not required according to EU-Directive 91/414/EEC because no direct application on water bodies is intended. Nevertheless, acute studies for the sediment dwelling species *Chironomus riparius* are available and a risk assessment is presented below.

Table IIIA1 10.2.1.5-1:Acute TER for *Chironomus riparius* exposed to spirotetramat and its metabolites

Test Organism, time scale	Test substance	Ecotoxicological endpoint	PEC_{max} [mg/L]	TER	Refine-ment required?
Spirotetramat					
Chironomus riparius static, 48 h, water-only	a.s.	LC_{50} 1.38 mg a.s./L	0.0116	**119**	**No**
Chironomus riparius static, 48 h, water-only	OD 150	LC_{50} 0.82 mg a.s./L	0.0116	**71**	**Yes**
BYI 08330-enol					
Chironomus riparius static, 48 h, water-only	metabolite	LC_{50} 74.9 mg p.m./L	0.0156	**4801**	**No**
BYI 08330-ketohydroxy					
Chironomus riparius static, 48 h, water-only	metabolite	LC_{50} > 100 mg p.m./L	0.0086	**>11628**	**No**
BYI 08330-methoxy cyclohexanone					
Chironomus riparius static, 48 h, water-only	metabolite	LC_{50} > 100 mg p.m./L	0.0014	**>71429**	**No**
BYI 08330-methoxycyclohexylaminocarboxylic acid					
Chironomus riparius static, 48 h, water-only	metabolite	LC_{50} > 100 mg p.m./L	0.0012	**>83333**	**No**

The acute TER values of *Chironomus riparius* exposed to spirotetramat and its metabolites are well above the trigger of 100. However, the trigger is not passed for the OD 150 formulation, based on worst case PEC values, so a refined risk assessment is presented.

Refined risk assessment

In a first step, the crop specific FOCUS STEP 2 PEC_{SW} values were considered for a more realistic TER calculation (see Table IIIA1 10.2.1-1).

Table IIIA1 10.2.1.5-2:Refined TER calculation for *Chironomus riparius* exposed to Spirotetramat OD 150 considering crop specific PECsw values according to FOCUS STEP 2

Test Organism	Application scenario	Distance from field [m]	Max. PECsw [mg/L]	LC_{50} [mg a.s./L]	TER	Refinement required?
Chironomus riparius	Citrus	0 m	0.0116	0.82	**71**	**Yes**
	Vegetables	0 m	0.0006		**1,367**	**No**

As indicated by the TER values, no buffer zone or further refinement is needed for application of Spirotetramat OD 150 in vegetables (lettuce) as the trigger value of 100 is clearly passed.

For application in citrus, PEC_{SW} values were calculated according to FOCUS STEP 3 and 4, where an exposure assessment using realistic worst-case scenarios is performed. These scenarios consider specific combinations of weather, soil, crop and water body and require the use of the deterministic models PRZM, MACRO and TOXSWA. The spray drift exposure can be significantly reduced by vegetated buffer zones (see [redacted], 2006, report MEF-06/281).

Table IIIA1 10.2.1.5-3: Refined TER calculation for *Chironomus riparius* exposed to Spirotetramat OD 150 considering maximum PECsw values for different buffer zones according to FOCUS STEP 3 and 4 for an application to citrus

Test Organism	Application scenario	Buffer zone	Max. PECsw [mg/L]	LC_{50} [mg a.s./L]	TER	Refinement required?
Chironomus riparius	Citrus	0 m	0.0084	0.82	**98**	**Yes**
		5 m	0.0059		**139**	**No**

As indicated by the TER values, for application in citrus, a buffer zone of 5 m may be considered to exclude unacceptable risks to aquatic invertebrates.

IIIA1 10.2.1.6 TER_{LT} for an aquatic insect species

The long-term TER for an aquatic insect species is not required according to EU-Directive 91/414/EEC because no direct application on water bodies is intended. However, a chronic study for the sediment-dwelling species *Chironomus riparius* is available and the TER_{LT} for an aquatic insect species is covered by the chronic TER for sediment-dwelling organisms.

Table IIIA1 10.2.1.6-1:Long-term TER for *Chironomus riparius* exposed to spirotetramat

Test Organism, time scale	Test substance	Ecotoxicological endpoint	PEC_{max} [mg/L]	TER	Refine-ment required?
Chironomus riparius static, 28 d, spiked water	a.s.	$EC_{15\,ER}$ 0.27 mg a.s./L $EC_{15\,DR}$ > 0.80 mg a.s./L	0.0116	**23** **> 69**	**No**

The TER value of spirotetramat is well above the Annex VI trigger value of ≥ 10. Thus, a refinement of the chronic *Chironomus riparius* risk assessment is not required.

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IIIA1 10.2.1.7TER$_A$ for an aquatic crustacean species

Not required according to SANCO/3268/2001 rev.4 (final, October 2002) since direct use in water bodies is not intended.

IIIA1 10.2.1.8TER$_{LT}$ for an aquatic crustacean species

Not required according to SANCO/3268/2001 rev.4 (final, October 2002) since direct use in water bodies is not intended.

IIIA1 10.2.1.9TER$_A$ for an aquatic gastropod mollusc species

Not required according to SANCO/3268/2001 rev.4 (final, October 2002) since direct use in water bodies is not intended.

IIIA1 10.2.1.10 TER$_{LT}$ for an aquatic gastropod mollusc species

An accepted international guideline for a chronic test on gastropods is currently not available. Furthermore, gastropod molluscs are generally significantly less sensitive than *Daphnia* (see SANCO/3268/2001 rev.4 (final, October 2002)). Moreover, no direct application on water bodies is intended. Thus, chronic studies with aquatic gastropod molluscs are not considered necessary.

IIIA1 10.2.1.11 TER$_{LT}$ for algae

The following TER calculations are based on the endpoints for spirotetramat, its metabolites and the most sensitive freshwater algae species *Pseudokirchneriella subcapitata* (see Table IIIA1 10.2-1).

Table IIIA1 10.2.1.11-1: Long-term TER for algae exposed to spirotetramat and its metabolites

Test Organism, time scale	Test substance	Ecotoxicological endpoint	PEC$_{max}$ [mg/L]	TER	Refine-ment required?
Spirotetramat					
Pseudokirchneriella subcapitata static, 72 h	a.s.	E_rC_{50} 8.15 mg a.s./L	0.0116	**703**	**No**
Pseudokirchneriella subcapitata static, 72 h	OD 150	E_rC_{50} > 8.2 mg a.s./L	0.0116	**> 707**	**No**
BYI 08330-enol					
Pseudokirchneriella subcapitata static, 72 h	metabolite	E_rC_{50} > 100 mg p.m./L	0.0156	**> 6410**	**No**
BYI 08330-methoxycyclohexanone					
Desmodesmus subspicatus static, 72 h	metabolite	E_rC_{50} > 100 mg p.m./L	0.0014	**>71,429**	**No**

The TER values for the tested algae species are well above the trigger value defined in Annex VI of Directive 91/414/EEC (TER ≥ 10). Thus, it can be concluded that no adverse effects on algae are to be

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expected from the use of Spirotetramat OD 150, spirotetramat tech. and its metabolites BYI 08330-enol and 4-Methoxycyclohexanone according to the proposed use pattern.

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IIIA1 10.2.2 Acute toxicity (aquatic) of the preparation

Acute toxicity for a representative species of aquatic insects (*Chironomus riparius*)

Report:	**KIIIA1 10.2.2/01, [redacted]; 2006**
Title:	Acute Toxicity of BYI 08330 OD 150 to Larvae of *Chironomus riparius* in a 48 h Static Laboratory Test System. Date: 2006-01-19
Organisation:	Bayer CropScience AG, [redacted], Germany
Report No.:	EBFNM007; M-264018-01-2
Publication:	unpublished
Dates of experimental work:	June 24, 2005 – June 29, 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. As primary endpoint, a concentration causing 50 percent mortality to larvae of *Chironomus riparius* (48 h - LC_{50}) has to be determined. Beside mortality a possible occurrence of symptoms was recorded and evaluated after 48 hours of exposure.
For this purpose, larvae of *Chironomus riparius* were exposed under defined conditions to the nominal concentrations 2.12, 3.71, 6.62, 11.9, 21.2, 37.1 and 66.2 mg formulation/L and compared against 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 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 99%) and the analytical findings after 2 days (average 75%), all results are based on nominal initial concentrations of the formulation.
The 48 h - LC_{50} was calculated by Logit Analysis to be 5.44 mg product/L (C.I. 95%: 3.45 – 7.68 mg product/L), corresponding to 0.82 mg a.s./L (C.I. 95%: 0.52 – 1.16 mg a.s./L).

Material and Methods

A Materials

1. Test material	BYI 08330 OD 150
Description	Light brown granule
Lot/batch No.	Batch no.: 08030/0189(0152) TOX no.: 07034-00;
Analysed content	148.89 g/L
Density	0.986 g/mL
Stability of test compound	Expiration date: 2006-03-10, when stored at room temperature
2. Vehicle and/or positive control	Elendt M7-medium, based on de-ionised water, was used to prepare the stock solution of the test item.
3. Test animals	
Species	*Chironomus riparius*
Age	1st instar-larvae, < 2-3 days old

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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 24, 2005 to June 29, 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 - 7 cm 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 66.4mg BYI 08330 OD 150 (nominally: 66.2 mg) in 1000 mL test medium.

The range of test concentrations was selected basing on pre-experiments or historical data: 0 (control), 2.12, 3.71, 6.62, 11.9, 21.2, 37.1 and 66.2 mg product/L (nominal), 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 except 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. The method was validated within the current study.

LC_{50} values and confidence intervals were calculated for the stated exposure period, using a commercial program (ToxRat Professional® Software, Vers.2.09).

RESULTS AND DISCUSSION

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A. Findings

Validity criteria were met as control mortality was not more than 10% (being 10%) 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 20.6°C to 21.4°C over the whole period of testing (light intensity at the beginning of the test 786 lux), the pH values ranged from 7.8 to 8.4.

The analytical findings of BYI 08330 found in all freshly prepared test levels on day 0 in reference to nominal concentrations ranged between 86 and 109% (average 99%). In aged test levels on day 2 analytical findings were between 68 and 80% (average 75%) 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 formulation.

Acute toxicity of BYI 08330 OD 150 to first instar-Larvae of *Chironomus riparius* after 48 hours (based on nominal concentrations)

Test Concentration (nominal) [mg product/L]	Exposed Chironomids (=100%)	Mortality after 48 h	
		n	%
Control	40	4	10
2.12	40	4	10
3.71	40	13	32.5
6.62	40	33	82.5
11.9	40	39	97.5
21.2	40	40	100.0
37.1	40	40	100.0
66.2	40	40	100.0

CONCLUSIONS

Based on nominal concentrations of the formulation, the 48 h- LC_{50} was calculated by Logit Analysis to be 5.44 mg product/L (C.I. 95%: 3.45 – 7.68 mg product/L), corresponding to 0.82 mg a.s./L (C.I. 95%: 0.52 – 1.16 mg a.s./L).

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

Report:	**KIIIA1 10.2.2.1/01, [redacted]; 2005**
Title:	Acute toxicity of BYI 08330 OD 150 to fish (*Oncorhynchus mykiss*) under static conditions Date: 2005-06-29
Organisation:	Bayer CropScience AG, [redacted], Germany
Report No.:	EBFNM008; M-253916-01-2
Publication:	unpublished
Dates of experimental work:	April 04, 2005 - April 12, 2005
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 OD 150 to Rainbow trout expressed as 96 h-LC_{50} for mortality. Ten fish in each test level were exposed for 96 h under static conditions to nominal concentrations of 2.50, 5.00, 10.0, 20.0 and 40.0 mg test item/L against a 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. Analytical determinations of BYI 08330 concentrations were made in the test media at the beginning of the test, after 48h and at the end of the test. Due to analytical measurement, the results of this study are given as nominal concentrations.

The 96 h - LC_{50} was calculated by logit analysis to be 9.48 mg test item L (C.I.95%: 5.84 - 15.7 mg/L), corresponding to 1.43 mg a.s./L, based on analysed content.

MATERIAL AND METHODS

A Materials

1. Test material	Spirotetramat (BYI 08330) OD 150
Description	Light brown suspension
Lot/batch No.	Batch no.: 08030/0189(0152) Tox no.: 07034-00
Analysed content	148.89 g/L (15.1%)
Stability of test compound	Not specified
2. Vehicle and/or positive control	-
3. Test animals	
Species	Rainbow trout (*Oncorhynchus mykiss*), mean body length 4.6 cm, mean body weight 0.8 g, biomass loading was 0.20 g fish/L test medium
Age	Juvenile
Source	[redacted], Germany.
Acclimation period	> 14 days acclimation period
Environmental conditions	
Temperature	11.6°C to 12.2°C
Photoperiod	16 hours light / 8 hours dark

B Study design and methods

1. In life dates	April 04, 2005 - April 08, 2005
2. Experimental treatments	

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Rainbow trout (*Oncorhynchus mykiss*), (lot F 2/05) were obtained from and identified by hatchery Fischzucht [redacted], Germany. 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 evaluated 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 ([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 2.50, 5.00, 10.0, 20.0 and 40.0 mg test item/L. An untreated water control was run in parallel. Test media were prepared immediately (about 30 min.) prior to the test.

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.

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 103% and 107% of nominal were found in all exposure levels over the whole testing period of 96 hours, thus, the results of this study are given as nominal concentrations.

The physical/chemical properties corresponded to the required values: dissolved oxygen concentrations ranged from 95 to 102% oxygen saturation, the pH values ranged from 6.9 to 7.2 and the water temperature ranged from 11.6°C to 12.2°C in all aquaria over the whole testing period.

There were neither any sublethal effects nor any mortality in the control group.

The minimum concentration causing 100% mortality (96 h) was 20.0 mg test item/L. The maximum concentration causing no mortality within the period of the test (NOLEC) was 2.50 mg test item/L. The no-observed-effect-concentration (NOEC) was 2.50 mg test item/L.

Exposure period (hours)	LC_{50} [mg test item/L]	95% C.I. [mg test item/L]	Method of statistical calculation
24	16.3	10.6 - 33.1	Logit analysis
48	14.9	9.85 - 27.9	Logit analysis
72	12.6	9.15 - 18.9	Logit analysis
96	9.48	5.84 - 15.7	Logit analysis

B. Observations

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Observations on the physical behaviour of the test item in the aquaria revealed a homogeneous dispersion in the water with turbidity observable at the two lowest concentrations of 2.50 and 5.00 mg test item/L. Intensive turbidity caused by the test item was observed at concentration ≥ 10 mg test item/L.

There were behavioural observations on fish caused by the test item over the whole exposure period in all test levels ≥ 5.0 mg test item/L. At the test level with 5.0 mg test item/L fish showed beside mortality the following symptoms after 96 h: laid inactive on the bottom of the aquarium; laid on their sides or backs; turned dark in coloration; showed laboured respiration; showed loss of equilibrium with lateral deviation from their normal orientation; were hyperactive - showed exaggerated response to stimulus or disturbance.

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

Nominal conc. [mg test item./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
2.50	10	N	10	N	10	N	10	N	10	N
5.00	10	N	10	N	0 1 2 6 1	TF BO,SR,AT,TS BO,AT OB N	0 2 4 4 0	TF BO,AT,DF,SR TS,AT H,AT N	1 3 4 2 0	TF BO,SR,DF,AT TS,AT H,AT N
10.0	10	N	0 1 6 3 0	TF BO,SR,AP, OB,AT AT N	0 5 2 3 0	TF BO,SR,AT,DF BO,SR,AT,TS TS,AT,DF N	1 9 0	TF BO,SR,DF,AT N	1 9 0	TF BO,SR,DF AT N
20.0	0 7 3	TF OB,AT N	7 2 1 0	TF BO,SR,KR,AT OB,SR,KR,AT,DF N	8 2 0	TF BO,KR,DF,AT,SR N	9 1 0	TF BO,SR,AT,DF N	10	TF
40.0	0 10 0	TF BO,SR,AT,KR,DF N	10	TF	-	-	-	-	-	-

Abbreviations:
AP: were inactive or displayed abnormally low activity
AT: showed labored 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
- no observations, all fish dead

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CONCLUSION

Based on nominal concentrations, the 96 h - LC_{50} was calculated by logit analysis to be 9.48 mg test item L (C.l. 95%: 5.84 - 15.7 mg/L), corresponding to 1.43 mg a.s./L, based on analysed content. The NOEC (highest concentration without sublethal effects) was considered to be 2.50 mg test item/L.

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

In tests with the active substance, *Daphnia* turned out to be the least sensitive species compared to fish algae and sediment dwelling organisms (see Table IIIA1 10.2-1). Considering the results for the OD 150 formulation with these species, the resulting endpoints were in the same range as for the active substance. Thus, the acute and chronic tests with spirotetramat tech. as test item are considered sufficient to assess the risk to *Daphnia* and no test with the formulation is deemed necessary.

IIIA1 10.2.2.3 Effects on algal growth and growth rate

Report:	**KIIIA1 10.2.2.3/01, [redacted]; 2006**
Title:	*Pseudokirchneriella subcapitata* Growth Inhibition Test with BYI 08330 OD 150 Date: 2006-01-16
Organisation:	Bayer CropScience AG, [redacted], Germany
Report No.:	EBFNM006; M-264263-01-2
Publication:	unpublished
Dates of experimental work:	May 27, 2005 – September 07, 2005
Guidelines:	Draft Proposal for Updating OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (October 22, 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 EC_x for growth rate of algal biomass (cells per volume). *Pseudokirchneriella subcapitata* were exposed in a multigeneration test for 3 days under static exposure conditions to the nominal concentrations of 9.53, 17.1, 30.9, 55.6 and 100 mg product (BYI 08330 OD 150)/L in comparison to control.

The pH values ranged from 8.0 to 8.9 in the controls, the incubation temperature and the continuous illumination corresponded to the aspired values. Quantitative amounts of BYI 08330 OD 150 were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period. All reported results are based on nominal test concentrations of the formulation (BYI 08330 OD 150), because the toxicity can only be attributed to the formulation as a whole.

The E_rC_{50} (0 - 72 h) was determined to be > 100 in mg product/L (corresponding to > 8.20 in mg geometric mean a.s./L), the LOE_rC (0 - 72 h) to be 55.6 in mg product/L (corresponding to 4.05 in mg geometric mean a.s./L) and the NOE_rC (0 - 72 h) to be 30.9 in mg product/L (corresponding to 2.02 in mg geometric mean a.s./L).

MATERIAL AND METHODS

A Materials

1. Test material	Spirotetramat as formulation BYI 08330 OD 150
Description	Light brown suspension

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Lot/batch No.	Batch no.: 08030/0189(0152) Tox no.: 07034-00
Analysed content	148.89 g a.s./L
Stability of test compound	2006-03-10
2. Vehicle and/or positive control	No positive control; formulation (oil based suspension) in solution of nutrient medium
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 [redacted] Germany. Transferred to the laboratory on July 15, 2002 and kept since then.
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	23 ± 2°C
Photoperiod	Continuous illumination, 4440 - 8880 lux (± 15%)

B Study design and methods

1. In life dates — May 27, 2005 to June 02, 2005
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 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 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, deionised water served as water source.

The stock solution was prepared immediately prior to the test, it contained 220.8 mg BYI 08330 OD 150 in 2210 g nutrient medium.

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), 9.53, 17.1, 30.9, 55.6, and 100 mg product/L (nominal initial), test duration was 3 days.

3. Observations

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

Morphological examination of cells by a microscope were made over the exposure period on each study day to detect possible alterations in algae cells such as unusual cell size. Cell numbers per volume (as a surrogate for biomass per volume) were estimated by direct algae cell counting under a microscope at a magnification of 400 times.

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For analytical verification samples were analysed for the actual concentration of BYI 08330 present in the test medium at all treatment levels and the control 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 given by the mentioned guideline.
The analytical findings of BYI 08330 OD 150 in the treatment levels found on day 0 were 91 to 96% of nominal (average 92.8%). On day 3 analytical findings of 19 to 31% of nominal (average 23.4%) were found. The low analytical findings on day 3 are explained by the hydrolytic half-life of BYI 08330 under alkaline conditions of testing.
The incubation temperature ranged from 22.3°C to 23.3°C over the whole period of testing at a continuous illumination of 6339 lux, the pH values extended from 8.0 to 8.9 in the controls.

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

Nominal Concentration [mg product./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]
control	642 000	1.383	--	0.501
9.53	667 000	1.386	-0.2	0.500
17.1	578 000	1.350	2.4	0.513
30.9	543 000	1.332	3.7	0.520
55.6	410 000	1.234	10.8	0.562
100	208 000	1.008	27.1	0.688

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

B. Observations

Morphological change in algae was observed in the test concentration of 100 mg product/L (some enlarged cells).

Conclusions

The E_rC_{50} (0 - 72 h) was determined to be > 100 in mg product/L (corresponding to > 8.20 in mg geometric mean a.s./L), the LOE_rC (0 - 72 h) to be 55.6 in mg product/L (corresponding to 4.05 in mg geometric mean a.s./L) and the NOE_rC (0 - 72 h) to be 30.9 in mg product/L (corresponding to 2.02 in mg geometric mean a.s./L).

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

This point is not an 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. For results with the active compound see IIA 8.11.1.

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

This point is not an 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.

IIIA1 10.2.3 Microcosm or mesocosm study

Not required due to the findings presented above.

IIIA1 10.2.4 Residue data in fish (long term)

Crop protection products with a high bioaccumulation potential (log P_{OW} > 3) could theoretically bear a risk of secondary poisoning for birds if contaminated prey like fish or earthworms are taken up.
Spirotetramat has a log P_{OW} value of 2.51 (pH 7) (see KIIA 2.8.1), indicating no relevant potential for bioaccumulation.
The spirotetramat metabolites BYI 08330-enol and BYI 08330-ketohydroxy have no potential to bioaccumulate as the log P_{OW} for both metabolites is < 3 (log P_{OW} = 1.3 at pH 7 for BYI 08330-ketohydroxy (see KIIA 7.13/05) and log P_{OW} = 0.3 at pH 7 for BYI 08330-enol, see KIIA 7.13/03). Bioconcentration studies to determine the BCF are therefore not deemed necessary.

IIIA1 10.2.5 Chronic fish toxicity data

For spirotetramat sufficient data are available to perform a risk assessment for fish on the basis of the data obtained from the respective Annex-points (IIIA1 10.2 and IIA 8.2). No chronic fish toxicity study has been conducted with Spirotetramat OD 150 since the product contains only one active substance and therefore the toxicity of the product can be predicted based on the toxicity of its active substance. Therefore conduction of such an additional study with higher animals has not been considered justified based on animal welfare reasons, as recommended in the EU Guidance Documents (SANCO/3268/2001 rev.4 (final, October 2002), section 8.2)

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

No study required, for explanation see IIIA1 10.2.5 above.

IIIA1 10.2.5.2 Fish early life stage toxicity test

No study required, for explanation see IIIA1 10.2.5 above. For results with the active substance see KIIA 8.2.4/01.

IIIA1 10.2.5.3 Fish life cycle test

No study required, for explanation see IIIA1 10.2.5 above.

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IIIA1 10.2.6 Chronic toxicity to aquatic invertebrates

For spirotetramat sufficient data are available to perform a risk assessment for aquatic organisms on the basis of the data obtained from the respective Annex-points (IIIA1 10.2 and IIA 8). Since no direct application on water bodies is intended, no further studies have been performed on aquatic invertebrates.

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

No study required, for explanation see IIIA1 10.2.6 above. For results with the active substance see KIIA 8.3.2.1/01.

IIIA1 10.2.6.2 Chronic toxicity for a representative species of aquatic insects

No study required, for explanation see IIIA1 10.2.6 above.

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

No study required, for explanation see IIIA1 10.2.6 above.

IIIA1 10.2.7 Accumulation in aquatic non-target organisms

Spirotetramat and its metabolites have log P_{OW} values < 3 (see KIIA 2.8.1), indicating no relevant potential for bioaccumulation in aquatic non-target organisms.

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IIIA1 10.3 Effects on terrestrial vertebrates other than birds

Table IIIA1 10.3-1 gives a summary of the results of the ecotoxicological relevant studies on mammals conducted with the active substance spirotetramat. All studies referred to herein have been conducted in compliance with the prevailing OECD or EPA testing guidelines and under GLP.

Comment: Detailed descriptions of toxicological studies are given in AII point 5 & AIII point 7.

Consideration of metabolites:

The following metabolites of spirotetramat were observed in plants metabolism studies at levels above 0.01 mg/kg: BYI 08330–enol , BYI 08330–enol glycoside., BYI 08330-ketohydroxy, BYI 08330-monohydroxy, BYI 08330-di-hydroxy, BYI 08330-desmethyl-enol, and BYI 08330-desmethyl-ketohydroxy .

The enol metabolite is also the primary and main metabolite in the rat and hen metabolism study ([redacted], J., [redacted], A., 2006, KIIA 6.2.2/01). In so far, it can be considered to be toxicologically well characterised and to be covered by the respective mammalian toxicological studies available which were conducted with the parent compound.

BYI 08330-desmethyl-enol was also observed in the rat and hen metabolism study and thus is covered by respective toxicological studies conducted with parent compound.

Likewise, the ketohydroxy metabolite was detected in the rat, and furthermore, an acute toxicity study on rat is available for this metabolite (see KIIA 5.8/01, [redacted], M., 2005) which reveals that this metabolite shows no toxicity to mammals (LD_{50} > 5000 mg a.s./kg bw). This is supported by the observation that the ketohydroxy metabolite proved less toxic than the parent compound and than the enol metabolite in all ecotoxicological studies conducted.

The metabolites BYI 08330-monohydroxy, BYI 08330-di-hydroxy and BYI 08330-desmethyl-ketohydroxy showed no toxicity in acute rat studies (LD_{50} > 5000 mg a.s./kg bw, see KIIA 5.8/05, [redacted], 2005, KIIA 5.8/07, [redacted], M., 2006 and KIIA 5.8/03, [redacted], M., 2006, respectively); it can thus be considered highly unlikely that exposure might result in unacceptable effects to terrestrial vertebrates.

The enol glycoside metabolite is immediately and quantitatively metabolised to BYI 08330-enol after dietary uptake (see IIA point 6). Thus, this metabolite can be considered to be ecotoxicologically sufficiently covered.

Thereby, the plant metabolites of spirotetramat are covered by existing ecotoxicology or toxicology studies, or they were shown not to be toxic to terrestrial vertebrates. In so far, it is justified to base the risk assessment on spirotetramat for birds on the parent compound.

Table IIIA1 10.3-1: Ecotoxicological endpoints for mammals (spirotetramat)

Test organisms	Duration	Test substance	Reference	Ecotoxicological endpoint	
Rat	acute	tech.	KIIA 5.2.1/01 [redacted], 2004	LD_{50}	> 2000 mg a.s./kg bw > 5000 mg a.s./kg bw (cut off value)
Rat	acute	OD 150	AT02161, [redacted], 2005	LD_{50}	> 5000 mg product/kg bw (equivalent to > 755 g a.s./kg bw*)
Rat	chronic		KIIA 5.6.1/02, [redacted], 2006	$NOAEL_{female}$	1000 ppm (equal to premating doses of 70.7 mg/kg bw/d)

*based on analytical content of 148.89 g/L

Default Values for Exposure Assessment

The default values for the acute and long-term exposure in the Tier 1 risk assessment are selected according to recommendations of the "Guidance Document on Risk Assessment for Birds and Mammals

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Under Council Directive 91/414/EEC", September 2002 (SANCO / 4145 /2000 – final). According to this guidance document, crop specific generic indicator species with specific daily food intake rates (FIR) related to the body weight (bw) should be used for the Tier 1 risk assessment. Spirotetramat OD 150 is an insecticide that is applied by spray application in leafy crops (lettuce) and in orchards (citrus). For spray applications in leafy crops the risk assessment should be based on generic data for medium herbivorous mammals (e.g. hare). For spray application in orchards small herbivorous mammals are used as model organisms. These generic data are summarised in Table IIIA1 10.3-2.

Table IIIA1 10.3-2: Exposure scenario for spray application in leafy crops and orchards– default values for acute- and long-term exposure. For explanation of the single terms see text below

Indicator species	Medium herbivorous mammal	Small herbivorous mammal
Crop scenario	Leafy crops	Orchards
Body weight (bw), indicator species [g]	3000	25
FIR: Food (fresh) intake rate [g/d]	832	34.8
FIR related to bw [g *feed*/g *bw*/d]	0.28	1.39
Acute toxicity RUD acute (default value) [(mg *residue*/kg *feed*)/(kg a.s./ha)]	87	85
Long-term exposure RUD long-term (default value) [(mg *residue*/kg *feed*)/(kg a.s./ha)]	40	46

- **FIR:** The daily intake of fresh food related to body weight is the quotient of food intake rate (**FIR**) and body weight.
- **RUD:** residue per unit dose. The RUD is an estimate of typical expected residues on food items [mg a.s./kg] normalised to an application rate of 1 kg a.s./ha. Different percentiles for the residue values are used for assessing different scenarios.
  For the acute risk assessment, it is assumed that a mammal is exposed to food items with residues at the upper end of the residue distribution, i.e. the 90%tile values are used for **RUD acute**.
  RUD long-term exposure: for long term exposure scenarios it is very unlikely that one individual will always be exposed to food contaminated with a high level of a plant protection compound. Due to the mobility of the animal, the arithmetic mean value of residues is the more appropriate worst case assumption for this exposure situation.
  All RUD data are taken from the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (SANCO/4145/2000-final).

IIIA1 10.3.1 Toxicity exposure ratios for terrestrial vertebrates other than birds

IIIA1 10.3.1.1 Acute toxicity exposure ratio (TER_A)

The TER figures are calculated on the basis of estimated theoretical exposure (ETE). According to the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" the ETE value for the acute risk assessment can be obtained with the following formula:

ETE (acute) = (FIR/bw) × RUD × application rate × MAF × PT × PD

with:

- RUD: RUD acute, see Table IIIA1 10.3-2
- MAF: Multiple Application Factor. In case of repeated applications, the MAF has to be taken into account for calculating the ETE for herbivorous mammals. The MAF is a function of the number of applications, interval and DT_{50}. In leafy crops (lettuce) Spirotetramat OD 150 is recommended to be applied at maximum 2 times. According to the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC", for 2 applications the default MAF (for an interval of 14 days as a worst case assumption covering also an interval of 21 d) is 1.2.
- PT: Fraction of diet obtained in treated area (number between 0 and 1)
- PD: Fraction of food type in diet (number between 0 and 1; one type or more types) In the first tier worst case approach, PT and PD are set to 100%.

The risk assessment for acute exposure is based on the endpoint for the active substance, since with the active substance higher a.s. concentrations were tested than with the formulation, where no adverse effects could be observed at the highest test concentration.

Table IIIA1 10.3.1.1-1: TER calculation based on acute toxicity and exposure to Spirotetramat OD 150 (use in leafy crops/orchards). Ecotoxicological Endpoint based on Rat

Application (Spirotetramat OD 150)	Leafy crops (lettuce)	Orchards (citrus)
Max. application rate [kg a.s./ha]	0.072	3 x 0.096 = 0.288
Indicator species	Medium herbivorous mammal	Small herbivorous mammal
Feed	Leafy crops	Short grass
FIR / bw [g *feed*/g *bw*/d]	0.28	1.39
RUD [(mg a.s./kg)/(kg a.s./ha)]	87	85
MAF (default)	1.2	1.2
ETE [mg a.s./kg/day]	2.1	40.8
LD_{50} [mg a.s./kg/day]	> 5000	> 5000
TER_A	> 2381	> 123
Refined Risk Assessment required	**No**	**No**

The acute risk assessment for mammals shows, that all TER-values are well above the trigger value according to Annex VI, 91/414/EEC (TER ≥ 10) even under the worst case assumptions of a Tier 1 risk assessment (see Table IIIA1 10.3.1.1-1). These results indicate a high margin of safety for mammals from the use of Spirotetramat OD 150 under practical conditions. Thus, no unacceptable acute risks to mammals are to be expected.

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

According to the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" version from September 2002 the short-term risk assessment for mammals is

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covered by the acute and long-term risk assessment. Thus, no TER_{ST} calculation and no short-term risk assessment are performed.

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

Ecotoxicologically relevant endpoint for chronic mammal toxicity

The "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (September 2002) states that the NOEL should be based on the most sensitive endpoint of relevance for survival rate, reproduction rate and development of individuals. These data can be derived best from rat multi-generation studies since this study type provides an option to follow up reproductive effects over more than one generation. For Spirotetramat, the NOAEL of the two-generation repro study is 70.7 mg/kg bw/d ([redacted], 2006, KIIA 5.6.1/02).

In a rabbit teratology study ([redacted], 2001, KIIA 5.6.11/02) a lower no effect level was found than in the rat two-generation study. At the level of 40 mg/kg bw/d, one abortion occurred in one single female individual (out of 24 individuals), as a consequence of body weight loss. This weight loss was not too severe (ca 250 g), and less pronounced as observed in the next higher treatment group (160 mg/kg bw/d), but triggered an abortion in this female.

Abortions as an unspecific reaction to loss of body weight are well known as a typical phenomenon specific to captive rabbits (see [redacted] et al.[8] (2005), M-274544-01-1) which are thus highly unlikely to occur in other mammal species or under field conditions. The conclusion that this abortion is specific to captive rabbits and should not be considered a primary reproductive effect is further supported by the fact that reproductive effects of BYI 08330 were not observed in mice or rat at comparable exposure levels.

Since the abortion observed at 40 mg/kg bw/d is clearly an isolated case of a secondary effect and specific to captive rabbits, it should not be extrapolated to other species, as it is done in ecotoxicological risk assessment. In so far, the only effect at this exposure level which needs to be considered for the ecotoxicological assessment of the results of this study is a loss of body weight in one female.

The primary focus of the chronic ecotoxicological risk assessment is on effects that are relevant at the population level. An incidental effect which was only seen in one out of 24 individuals of a treatment group, and which is manifested as a not too severe body weight loss without being a primary reproductive effect, cannot be considered as being relevant at the population level. In so far, the effect observed in the 40 mg/kg bw/d group should not be considered for the definition of the ecotoxicologically relevant LOAEL for chronic mammal risk assessment. The lowest dose at which clear ecotoxicologically relevant effects were seen in this study is the next higher tested dose, 160 mg/kg bw/d.

It should furthermore be noted that the route of application in the rabbit teratology study (gavage study with bolus application) was unrealistic in terms of exposure under field conditions. In so far, the significance of this study for the ecotoxicological risk assessment is limited.

The lowest chronic endpoint found in other ecotoxicologically relevant mammal studies was the aforementioned NOAEL of the rat two-generation reproduction study (70.7 mg/kg bw/d). This endpoint

[8] G.D. [redacted], T.L. [redacted], R.E. [redacted] and M.E. [redacted], 2005: Effects of Feed Restriction During Organogenesis on Embryo-Fetal Development in Rabbit, Birth Defects Research (Part B) 74: 424-430

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is higher than the highest dose tested in the rabbit teratology study at which no ecotoxicologically relevant effects were seen, but still significantly lower than the ecotoxicologically relevant LOAEL of the rabbit teratology study.
In so far, it is justified to consider the NOAEL of the rat two-generation reproduction study the most relevant endpoint for the chronic mammal risk assessment.

Risk assessment

According to the Guidance Document on Risk Assessment for Birds and Mammals, the estimated theoretical exposure (ETE) for the long-term TER determination has been calculated, based on the daily dietary dose, with the following formula:

ETE (long-term) = (FIR/bw) × RUD × application rate × MAF × twa-factor× PT × PD

with:

- RUD: RUD long-term, see Table IIIA1 10.3-2
- MAF: According to the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (September 2002) the default MAF (default DT_{50} of 10 days) for 2 applications in lettuce (interval of 14 days) is 1.4 and for 2 application in citrus orchards (interval of 21 days) is 1.2.
- twa-factor: The time-weighted average factor (f_{twa}) accounts for the average concentration of the residues during a certain time interval relative to the initial concentration. The default twa-factor according to the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC", is 0.53, assuming a DT_{50} on herbage of 10 days (= default).
- In the Tier 1 worst case approach, PT and PD are set to 100%.

These values represent a highly conservative worst case approach concerning the long term exposure of mammals. In order provide a still conservative but more realistic risk assessment the following parameters were used for the TER calculation:

<u>More realistic crop interception/more realistic RUD</u>
Spirotetramat is intended to be used as spray application in orchards. Citrus trees are sprayed by orchard sprayers. After spraying, most of the applied plant protection product adheres to the tree leaves, however, a certain proportion of the applied product reaches the soil surface and with it the herbs growing underneath the trees (which may serve herbivorous mammals as food source).
As a default value for insecticidal products, the Guidance Document (SANCO/4145/2000-final) propose a default crop interception factor of 0.4 (i.e. only 40% is adherent to the crop and 60% reaches the ground), resulting in the default "orchard" RUD for short grass of 46 (i.e. 0.6 × 76). However, this default value is mainly relevant to deciduous trees where degree of interception is depending on the development stage of the plants. Citrus, in contrast, is bearing leaves all around the year, which ensures a permanently high degree of interception.
This is more realistically taken into account in the provisions for crop interception as outlined in FOCUS Groundwater, where the crop-interception-tables for "citrus" consider at all growth stages a crop interception of actually 70%, rendering the percentage of the product reaching the ground to be only 30% (see also [redacted], 2006, Report No. MEF-06/282). Thus, considering the actual crop interception in citrus orchards at the time of Spirotetramat OD 150 application, the standard **RUD** for short grass (i.e. 76) can be refined in sensu SANCO/4145/2000-final for orchards to 0.3 × 76 = **22.8**.

<u>More realistic MAF/ twa-factor</u>
In the study of [redacted] et al. (2006, KIIIA1 10.1.7/01), the half life of spirotetramat on leaves of soybean, shortgrass, and tallgrass were determined. Mean half-life for soybean was 3.42 days. The half-life on short-grass or tallgrass was even shorter. Considering now soybean as a model plant for the type of diet

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relevant for herbivorous mammals in vegetable crops (non-grass herbs), a MAF of 1.06 (for lettuce) and of 1.01 (for citrus) and a 14-d twa factor of 0.33 can be derived according to the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (SANCO/4145/2000-final).

Table IIIA1 10.3.1.3-1: TER calculation based on long-term toxicity and exposure to Spirotetramat OD 150 (use in leafy crops/orchards). Ecotoxicological Endpoint based on rat

Application (Spirotetramat OD 150)	Leafy crops (lettuce)	Orchards (citrus)
Max. application rate [kg a.s./ha]	0.072	3 x 0.096 = 0.288
Indicator species	Medium herbivorous mammal	Small herbivorous mammal
Feed	Leafy crops	Short grass
FIR / bw [g *feed*/g *bw*/d]	0.28	1.39
RUD [(mg a.s./kg)/(kg a.s./ha)]	40	22.8*
MAF	1.06*	1.01*
twa-factor	0.33*	0.33*
ETE [mg a.s./kg/day]	0.3	3.0
NO(A)EL [mg a.s./kg/day]	70.7	
TER_{LT}	236	24
Refined Risk Assessment required	**No**	**No**

* refined values, for explanation see above

The Tier 1 long-term risk assessment for mammals shows that the TER_{LT}-value for medium herbivorous mammals in leafy crops and for small herbivorous mammals in orchards is higher than the trigger value of 5. A further refinement is hence not necessary.

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IIIA1 10.3.2 Effects on terrestrial vertebrates other than birds

IIIA1 10.3.2.1Acute oral toxicity of the preparation

For a detailed description see Spirotetramat OD 150, IIIA1, 7.1.1.

IIIA1 10.3.2.2Acceptance of bait, granules or treated seed

Not relevant as the product will be used as spray application.

IIIA1 10.3.2.3Effects of secondary poisoning

Crop protection products with a high bioaccumulation potential (log P_{OW} > 3) could theoretically bear a risk of secondary poisoning for mammals if contaminated prey like fish or earthworms are taken up.
For spirotetramat, the low log P_{OW} of 2.51 (see KIIA 2.8.1) indicates that a significant accumulation in potential prey organisms has not to be expected. Thus, based on the low log P_{OW} of the active substance, a risk assessment to account for secondary poisoning is not considered necessary.
This applies also to the metabolites of spirotetramat as the log P_{OW} of BYI 08330-cis-ketohydroxy is 1.3 (see KIIA 7.13/05) and of BYI 08330-enol is 0.3 (at pH 7, see KIIA 7.13/02). For the metabolites BYI 08330-Methoxycyclohexanone and BYI 08330-Methoxycyclohexylaminocarboxylic acid log P_{OW} values of 0.29 and - 2.02 were estimated with EPA program KOWWIN v1.67.

IIIA1 10.3.3 Supervised cage or field trials or other appropriate studies

Not necessary in view of the findings presented above.

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IIIA1 10.4 Effects on bees

The ecotoxicological endpoints of honey bee laboratory studies are provided in the corresponding Annex II dossier. In this document, the standard honey bee risk assessment, the results of higher tier studies as well as a crop specific risk assessment is presented.

Table IIIA1 10.4-1: Overview on higher tier studies

Test substance	Crop / Study Type / Result	Reference
Bee brood feeding studies (Annex II)		
Spirotetramat OD 100	Brood feeding test according to Oomen et al. (1992), sugar solution (0.0144% a.s.): Effects on brood were detected	[redacted], 2004 M-000345-01-2 KIIA 8.7.4/01
Spirotetramat SC 240	Brood feeding test according to Oomen et al. (1992), sugar solution (0.0144% a.s.): Effects on brood were detected	[redacted], 2004 M-121877-01-2 KIIA 8.7.4/02
Pilot research (orientating) studies		
Spirotetramat	Summary of orientating research tunnel trials on effects to honey bees (Non-GLP, Phacelia): Transient slight - medium brood effects were observed.	[redacted], 2007 M-294216-01-1 KIIIA1 10.4.4/01 (also filed KIIIA1 10.4.7/02)
Feeding studies		
Spirotetramat + Spirotetramat -enol	Honey bee colonies fed with spiked pollen, nominally 2, 10 and 20 mg total a.s./kg diet: No adverse effects on brood and brood development were found after feeding small honey bee colonies for 21 days with pollen spiked with a nominal concentration of up to and including 20 mg spirotetramat + spirotetramat-enol/kg pollen. There were no adverse effects on comb development, hive weight development, honey and pollen storage behaviour and foraging activity. No effect on adult or pre-imaginal mortality was found.	[redacted] *et al.*, 2008 M-306791-01-1 KIIIA1 10.4.6/01
Spirotetramat + Spirotetramat -enol	Honey bee colonies fed with spiked pollen, nominally 0.05, 0.2, 0.5, 2 and 10 mg total a.s./kg diet: A slight transient effect to larval abundance in the highest treatment group cannot be excluded, though data are not unequivocal. In the other treatment groups, no consistent effects were seen in this endpoint. There were no adverse effects to other brood-related endpoints, comb development, hive weight development, honey and pollen storage behaviour, foraging activity and mortality by foraging on and consumption of pollen containing up to 10 mg spirotetramat + spirotetramat -enol/kg pollen	[redacted] *et al.*, 2007 M-292891-01-1 KIIIA1 10.4.6/02

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Table IIIA1 10.4-1: Overview on higher tier studies (continued)

Test substance	Crop / Study Type / Result	Reference
Semi-field studies		
Spirotetramat OD 100	Oilseed rape (OSR), tunnel test with two treatment groups, T1: 3 × 72 g a.s./ha pre-flowering [7 d application interval] + 2 × 96 g a.s./ha [6 d application interval] during flowering; T2: 1 × 96 g a.s./ha during flowering: Both test item treatments (T1 and T2) did not result in an adverse effect on the mortality of adult honey bees, bee brood, flight intensity and behaviour of the bees in the crop area or in front of the hive.	[redacted], 2005 M-244490-01-1 KIIIA1 10.4.7/01
Spirotetramat OD 150	Strawberry, glasshouse test with one treatment group, T: 1 × 100 g a.s./ha during flowering: The test item treatment revealed no adverse effects on adult mortality, pupal mortality, egg laying activity, larval and pupal abundance, colony strength, hive development as well as on nectar and pollen storage	[redacted] *et al.*, 2009 M-354707-01-1 KIIIA1 10.4.7/03
Spirotetramat SC 100	Raspberries, tunnel test with one treatment group, T: 1 × 100 g a.s./ha during flowering: The test item treatment revealed slight to medium transient brood effects. No adverse effects on the mortality of adult honey bees, flight intensity and honey bee behaviour were observed.	[redacted], 2010 M-369450-01-1 KIIIA1 10.4.7/04
Field studies		
Spirotetramat OD 150	Phacelia, field study with two treatment groups, T1: 2 × 72 g a.s./ha pre-flowering [7 d application interval] + 2 × 72 g a.s./ha [7 d application interval] during flowering; T2: 2 × 96 g a.s./ha [7 d application interval] during flowering: Both test item treatments (T1 and T2) did not result in an adverse effect on honey bees as determined by mortality, flight intensity, behaviour as well as by colony strength, size of the brood nest and brood status.	[redacted], 2006 M-277194-02-2 KIIIA1 10.4.5/01
Spirotetramat OD 150	Citrus, field study with one treatment group, T: 1 × 96 g a.s./ha/m canopy height (corresponding to overall 1 × 192 g a.s./ha) pre-flowering + 1 × 96 g a.s./ha/m canopy height (corresponding to overall 1 × 192 g a.s./ha) during flowering [duration between the two applications: 21 d]: The test item treatment did not result in an adverse effect on honey bee mortality, flight intensity, behaviour, colony strength, size of the brood nest and brood development.	[redacted], 2008 M-307363-01-2 KIIIA1 10.4.5/04
Spirotetramat OD 150	Citrus, field study with one treatment group, T: 2 × 96 g a.s./ha/m canopy height (corresponding to total application rates of 173 - 278 g a.s./ha, depending on actual tree height) during flowering [14 - 16 d application interval]: The test item treatment did not result in an adverse effect on brood development (eggs, larvae, pupae) & abundance of adult honey bees. In addition, no effects on foraging activity, mortality as determined in front of the hives, hive weight development and the food storage behaviour of exposed honey bee colonies were found.	[redacted] *et al.*, 2010 M-363607-01-3 KIIIA1 10.4.5/05

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Table IIIA1 10.4-1: Overview on higher tier studies (continued)

Test substance	Crop / Study Type / Result	Reference
Field studies		
Spirotetramat SC 240	Citrus, field study with one treatment group, T: 1 × 175 g a.s./ha during flowering: The test item treatment did not result in an adverse effect on colony strength, colony health, brood cohort success, intra-hive mortality and hive weight change.	[redacted] *et al.*, 2010 M-386205-01-1 KIIIA1 10.4.5/06
Spirotetramat OD 150	Melon (flowering and bee-attractive vegetable crop), field study with two treatment groups, T1: 4 × 72 g a.s./ha during flowering [7, 8, 12 d intrval], T2: 4 × 88 g a.s./ha during flowering [7, 8, 12 d intrval]: Both test item treatments (T1 and T2) did not result in an adverse effect on brood development (eggs, larvae, pupae) and abundance of adult honey bees. In addition, no effects on foraging activity, mortality as determined in front of the hives, hive weight development and the storage behaviour of honeybee colonies were found. However, due to a certain uncertainty about exposure in the period after the 2nd application, a positive proof of the absence of effects in spite of full exposure to the treated crop is only given for the study period until the 1st assessment.	[redacted] *et al.*, 2008 M-301729-01-1 KIIIA1 10.4.5/03
Spirotetramat OD 150	Melon (flowering and bee-attractive vegetable crop), field study with two treatment groups, T1: 4 × 75 g a.s./ha during flowering [7 d intrval], T2: 2 × 88 g a.s./ha during flowering [7 d intrval]: Both test item treatments (T1 and T2) did not result in an adverse effect on brood development (eggs, larvae, pupae) and abundance of adult honey bees. Moreover, no adverse effects on foraging activity, mortality determined in front of the hives, hive weight development and the storage behaviour of honey bee colonies were found.	[redacted] *et al.*, 2008 M-303607-01-1 KIIIA1 10.4.5/02
Spirotetramat SC 100	Strawberry, field study with one treatment group (comprising two spatially separated replicates), T: 2 × 100 g a.s./ha during flowering [14 d intrval]: The test item treatment did not result in adverse effects on mortality, flight intensity, behaviour, colony status, brood development, strength and size of the brood nest. Ther was no evidence of disturbance or termination of the brood development.	[redacted], 2011 M-401434-01-1 KIIIA1 10.4.5/07

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Table IIIA1 10.4-1: Overview on higher tier studies (continued)

Test substance	Crop / Study Type / Result	Reference
Residues in bee-relevant matrices		
Spirotetramat OD 150	Citrus, residues in blossoms after spray application, 1 × 75 g a.s./ha/m canopy height pre-flowering + 1 × 75 g a.s./ha/m canopy height during flowering	[redacted], 2007 M-295273-01-1 KIIIA1 10.4.6.2/03
Spirotetramat OD 150	Citrus, residues in blossoms after spray application, 1 × 75 g a.s./ha/m canopy height pre-flowering + 1 × 75 g a.s./ha/m canopy height during flowering	[redacted], 2007 M-295276-01-1 KIIIA1 10.4.6.2/04
Spirotetramat OD 150	Citrus, residues in blossoms, nectar and pollen after spray application, 1 × 96 g a.s./ha/m canopy height pre-flowering + 1 × 96 g a.s./ha/m canopy height during flowering	[redacted], 2008 M-307363-01-1 KIIIA1 10.4.5/04
Spirotetramat OD 150	Citrus, residues in blossoms after spray application, 2 × 96 g a.s./ha/m canopy height during flowering	[redacted] *et al.*, 2010 M-363607-01-3 KIIIA1 10.4.5/05
Spirotetramat SC 240	Citrus, residues in blossoms, nectar and pollen after spray application, 1 × 175 g a.s./ha during flowering	[redacted] *et al.*, 2010 M-386205-01-1 KIIIA1 10.4.5/06
Spirotetramat OD 150	Melon (flowering and bee-attractive vegetable crop), residues in blossoms after spray application, 1 × 288 g a.s./ha or 1 × 72 g a.s./ha, sequential blossom sampling, pre-flowering and during flowering applications	[redacted] *et al.*, 2006 M-298511-01-1 KIIIA1 10.4.6.2/01
Spirotetramat OD 150	Melon (flowering and bee-attractive vegetable crop), residues in nectar and pollen after spray application, 1 × 288 g a.s./ha pre-flowering or 1 × 96 g a.s./ha during flowering	[redacted] *et al.*, 2006 M-298516-01-1 KIIIA1 10.4.6.2/02
Spirotetramat SC 100	Strawberry, residues in blossoms, nectar and pollen after spray application, 2 × 100 g a.s./ha during flowering	[redacted], 2011 M-401434-01-1 KIIIA1 10.4.5/07
Spirotetramat SC 100	Raspberry, residues in blossoms, nectar and pollen after spray application, 1 × 100 g a.s./ha during flowering	[redacted], 2010 M-369450-01-1 KIIIA1 10.4.7/04
Spirotetramat OD 150	*Phacelia*, residues in nectar and pollen after spray application, 1 × 100 g a.s./ha during flowering	[redacted] *et al.*, 2007 M-295137-01-1 KIIIA1 10.4.6.2/05
Spirotetramat OD 150	Oilseed rape (OSR), residues in nectar and pollen after spray application, 1 × 100 g a.s./ha during flowering	[redacted] *et al.*, 2007 M-295271-01-1 KIIIA1 10.4.6.2/06

Ecotoxicological endpoints

Table IIIA1 10.4-2: Ecotoxicological endpoints for bees

Test organisms	Duration	Test substance	Reference	Ecotoxicological endpoint	
Honey bee	acute, 48 h	techn.	KIIA 8.7.1/01	LD_{50} oral	107.3 µg a.s./bee
				LD_{50} contact	**> 100.0 µg a.s./bee**
Honey bee	acute, 48 h	OD 150	KIIIA1 10.4.2/01	LD_{50} oral	**91.7 µg a.s./bee**
				LD_{50} contact	162.0 µg a.s./bee

Endpoints used for risk assessment are marked in **bold**

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IIIA1 10.4.1 Hazard Quotients for bees

An indication of hazard (Hazard Quotient or Q_H) can be derived according to the EPPO risk assessment scheme, by calculating the ratio between the application rate (expressed in g/ha) and the lowest laboratory contact and oral LD_{50} (expressed in µg/bee).
Q_H values can be calculated using data from the studies performed with the active substance or with the actual formulation. Q_H values higher than 50 are assumed to reflect levels of concern which trigger higher tiered tests for clarification of the risk to honey bees.

Hazard Quotient, oral: $$Q_{HO} = \frac{\text{maximum application rate}}{LD_{50}\ \text{oral}} = \frac{[\text{g a.s./ha}]}{[\mu\text{g a.s./bee}]}$$

Hazard Quotient, contact: $$Q_{HC} = \frac{\text{maximum application rate}}{LD_{50}\ \text{contact}} = \frac{[\text{g a.s./ha}]}{[\mu\text{g a.s./bee}]}$$

IIIA1 10.4.1.1 Oral exposure Q_{HO}

Table IIIA1 10.4.1.1-1: Hazard quotients for bees exposed to spirotetramat – oral toxicity [1]

Crop	Exposure route	LD_{50} [µg/bee][9]	Appl. rate [g a.s./ha]	Hazard quotient Q_{HO}	Trigger value for higher-tier	A-priori acceptable risk for adult bees
Citrus	oral	91.7	288 [a)]	3.1	50	Yes
Lettuce	oral	91.7	72	0.8	50	Yes

[a)] 96 g a.s./ha/m canopy height, max. 3m height → maximum total application rate = 288 g a.s./ha, spray application (OD 150)

The hazard quotient for oral exposure is below the trigger value of 50 (i.e. $Q_{HO} < 50$). Thus, no unacceptable acute risk to adult honey bees is to be expected when the product is used according to the proposed use pattern.

Table IIIA1 10.4.1.1-2: Hazard quotients for bees exposed to spirotetramat – contact toxicity [1]

Crop	Exposure route	LD_{50} [µg/bee]	Appl. rate [g a.s./ha]	Hazard quotient Q_{HC}	Trigger value for higher-tier	A-priori acceptable risk for adult bees
Citrus	contact	> 100	288 [a)]	< 2.9	50	Yes
Lettuce	contact	> 100	72	< 0.7	50	Yes

[a)] 96 g a.s./ha/m canopy height, max. 3m height → maximum total application rate = 288 g a.s./ha, spray application (OD 150)

The hazard quotient for contact exposure is below the trigger value of 50 (i.e. $Q_{HC} < 50$). Thus, no unacceptable acute risk to adult honey bees is to be expected when the product is used according to the proposed use pattern.

[9] Honey bees have additionally been acutely tested with four metabolites of spirotetramat (SPT-cis-enol, SPT-cis-ketohydroxy, SPT-enol-glucoside and SPT-mono-hydroxy. All tests revealed LD_{50}–values of > 100 µg a.s./bee (acute contact and acute oral toxicity test). Study summaries are filed in Annex II, point IIA 8.7.1

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Risk posed by spirotetramat to honey bee brood

As the Q_{HO} and the Q_{HC} of spirotetramat is substantially below 50, when considering the maximum recommended field rate, an unacceptable acute risk for adult honey bees is a-priori not to be expected from contact or oral exposure. A further consideration of risks to adult honey bees is therefore not required.

However, due to its mode of action (inhibition of acetyl-CoA carboxylase, a key enzyme in the fatty acid biosynthesis), spirotetramat has the intrinsic property to affect insect larval development and as such also the larval development of honey bee brood. Any intrinsic toxicity property of a test compound on honey bee larvae can be detected in studies following the guideline for bee brood feeding tests according to Oomen et al., 1992 (EPPO Bulletin 22, 613-616). Two such bee brood feeding tests were conducted with spirotetramat, one with the representative formulation Spirotetramat OD 100 ([redacted], 2004; KIIA 8.7.4/01; Doc.-No.: M-000345-01-2), the other with the representative formulation Spirotetramat SC 240 ([redacted], 2004; KIIA 8.7.4/02; Doc.-No.: M-121877-01-1). In both bee brood feeding tests, brood effects were recorded after a sugar solution containing 0.0144% of the test item (a.s.) was fed to honey bee colonies. Based on the provisions of the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002-final), the bee brood feeding test represents a worst case test for highlighting intrinsic toxicity properties of a test compound but is not indicative for the risk potential under realistic use conditions. The characterization of the risk profile under exposure conditions representative for typical agronomic use conditions require a more sophisticated test design.

Higher tier honey bee studies and risk assessment for spirotetramat

In pilot studies under confined conditions, where spirotetramat was generally applied during the full-flowering period of the highly bee attractive surrogate crop *Phacelia* (Lacy Phacelia) and where honey bees were actively foraging on this crop, slight to medium transient brood effects were observed ([redacted], 2007; KIIIA1 10.4.4/01; Doc.-No.: M-294216-01-1). These effects were highly transient in nature and were no longer evident at the end of the respective study, thus, neither jeopardize colony vitality nor colony survival. In many cases, effects were so minor that considering the intrinsic variability of this endpoint, i.e. brood development, treatment-related effects were difficult to conclude. Since, however, there is apparently a potential of spirotetramat to cause bee brood effects, at least under worst-case exposure conditions (e.g. confinement or feeding with excessive dietary exposure levels), this risk potential was further investigated simulating various exposure scenarios which may occur in agronomic practice.

Forced exposure conditions (confinement / semi-field)

Two forced-feeding studies with spirotetramat (spirotetramat + spirotetramat-enol) were conducted under confined conditions ([redacted] *et al.*, 2007; KIIIA1 10.4.6/02; M-292891-01-1 and [redacted] *et al.*, 2008; KIIIA1 10.4.6/01; M-306791-01-1), in order to investigate whether pollen (for the nutrition of bee larvae, pollen plays a particularly important role), spiked with a mixture of spirotetramat and spirotetramat-enol, causes brood effects, when small honey bee colonies are fed with this spiked pollen under confined conditions for a period of one honey bee brood cycle (un-treated honey was offered as a carbohydrate source). No adverse effects on bee brood or any other investigated endpoint was concluded from both studies for all concentrations tested up to and including nominally 20 mg/kg pollen (analytically verified 14 mg total spirotetramat (= parent + enol metabolite)/kg pollen).

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In residue studies, after a spray application of spirotetramat to the full-flowering and highly bee attractive surrogate crops oil-seed rape ([redacted], 2007; KIIIA1 10.4.6.2/06; M-295271-01-1) and *Phacelia* ([redacted], 2007; KIIIA1 10.4.6.2/05; M-295137-01-1), at application rates corresponding to 1 × 100 g a.s./ha, respectively, residue levels of max. 7.7 mg/kg in pollen and of max. 0.3 mg/kg in nectar were found (Table IIIA1 10.4.1.1-3).

Table IIIA1 10.4.1.1-3: Residue levels of spirotetramat (parent + spirotetramat-enol) in bee-relevant matrices of treated crops after application (confined conditions)

Crop, application rate/ matrix	Residues in blossoms [mg/kg]	Residues in pollen [mg/kg]	Residues in nectar [mg/kg]	Origin
Oilseed rape (OSR), 1 × 100 g a.s./ha during flowering (tunnel)	n.a.	5.47 - 7.67	0.04 - 0.06	[redacted] *et al.*, 2007 M-295137-01-1 KIIIA1 10.4.6.2/05
Phacelia, 1 × 100 g a.s./ha during flowering (tunnel)	n.a.	2.12 - 6.12	0.20 - 0.31	[redacted] *et al.*, 2007 M-295271-01-1 KIIIA1 10.4.6.2/06

n.a.: not assessed

The maximum residue levels (ca. 6 - 8 mg/kg; see Table IIIA1 10.4.1.1-3) after one application of 100 g a.s./ha to full-flowering OSR and *Phacelia*, respectively, were below the highest concentration which has been tested in the above mentioned forced pollen feeding studies without adverse effects on colony vitality and brood development (ca. 14 mg/kg, see above). However, since the orientating pilot studies on flowering *Phacelia* revealed slight to medium transient brood effects at application rates ≤ 100 g a.s./ha under confined, but not agronomically atypical exposure conditions ([redacted], 2007; KIIIA1 10.4.4/01; Doc.-No.: M-294216-01-1), further higher-tier studies were performed to scrutinize the indicative power of the forced-feeding pollen study.

A semi-field brood test (confined conditions; [redacted], 2005; KIIIA1 10.4.7/01; M-244490-01-1) has been conducted with a representative formulation (Spirotetramat OD 100) in a bee attractive surrogate crop (spring oilseed rape) under confined conditions. This study design is recommended as a higher tier study approach if a potential for adverse effects on honey bee larvae is recorded in tests according Oomen *et al.*, 1992. In this study, spirotetramat was applied with three pre-flowering applications corresponding to 72 g a.s./ha each, followed by two applications of 96 g a.s./ha into the flowering crop during bee flight. This study did neither indicate treatment-related effects on bee brood and colony development nor effects on other endpoints, as adult mortality, foraging activity, or bee behaviour.

In a glasshouse study with flowering strawberries (confined conditions; [redacted] *et al.*, 2009; KIIIA1 10.4.7/03; M-354707-01-1), conducted with Spirotetramat OD 150 (1 × 100 g a.s./ha during flowering), no adverse effects on adult mortality, pupal mortality, egg laying activity, larval and pupal abundance, colony strength, hive development as well as on nectar and pollen storage have been detected.

In a further semi-field study with flowering raspberries (confined conditions; [redacted], 2010; KIIIA1 10.4.7/04; M-369450-01-1), conducted with Spirotetramat SC 100 (1 × 100 g a.s./ha during flowering), slight to medium transient brood effects have been detected, however, no adverse effects on the mortality of adult honey bees, flight intensity and honey bee behaviour were observed.

Overall, it can be concluded that under forced (confined) exposure conditions, when spirotetramat is applied via foliar application to bee-attractive, flowering crops, slight to medium transient brood effects cannot be excluded. However, the available studies further revealed that if brood effects occur, these effects do neither jeopardize colony vitality nor colony survival.

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Field studies

In order to investigate whether under field conditions the slight to medium transient brood effects which have been observed under forced exposure conditions are still detectable and whether there are indictions that applications of spirotetramat interfere with bee keeping activities under typical commercial use conditions, several field studies have been conducted in a range of model and target crops.

A field study with special focus on brood effects was conducted with the OD 150 formulation ([redacted], 2006; KIIIA1 10.4.5/01; M-277194-02-2). The study was conducted in the highly bee attractive surrogate crop *Phacelia*. Two application scenarios were tested, both with applications into the full-flowering crop, (i) 2 × 72 g a.s./ha during pre-flowering + 2 × 72 g a.s./ha during flowering and during foraging activity of the bees, (ii) 2 × 96 g a.s./ha during flowering and during foraging activity of the bees. No adverse effects were detected, neither on brood development or colony condition, nor on any other parameter assessed, such as mortality or foraging activity.

Moreover, in total three independent field studies have been conducted in citrus, accounting for one or two sequential spirotetramat applications during flowering, with actively foraging honey bees within medium-sized to large scale citrus plantations and application rates of up to and including 96 g a.s./ha/m canopy hight, which resulted in effective ha-based application rates of up to and including 278 g a.s./ha ([redacted], 2008; KIIIA1 10.4.5/04; M-307363-01-1; [redacted] et al., 2010; KIIIA1 10.4.5/05; M-363607-01-3; [redacted] et al., 2010; KIIIA1 10.4.5/06; M-386205-01-1). In none of the three field studies, where spirotetramat has been applied during the citrus flowering period, adverse effects on honey bee brood development, honey bee colony performance and well as on the storage behaviour of honey bee colonies have been observed. Moreover, in none of the three citrus-studies, adverse effects on honey bee mortality, foraging activity and honey bee behaviour have been detected.

Further, two independent field studies have been conducted in melons, which can be considered to be a representative flowering and bee-attractive vegetable crop ([redacted] *et al.*, 2008; KIIIA1 10.4.5/03; M-301729-01-1; [redacted] *et al.*, 2008; KIIIA1 10.4.5/02; M-303607-01-1). The tested application scenarios accounted for multiple applications during the flowering period, actively foraging honey bees and fully-exposed colonies at (i) 2 × 88 g a.s./ha and (ii) 4 × 75 g a.s./ha. In none of the two studies, adverse effects on honey bee brood development, honey bee colony performance and well as on the storage behaviour of honey bee colonies have been observed. Moreover, in none of the two melon studies, adverse effects on honey bee mortality, foraging activity and hive weight development have been detected.

In a strawberry field study ([redacted], 2011; KIIIA1 10.4.5/07; M-401434-01-1), spirotetramat was applied twice during bee-flight at a rate corresponding to 100 g a.s./ha, respectively (i.e. 2 × 100 g a.s./ha). The study comprised two spatially separated replicates with the corresponding applications carried out during the full-flowering period of the strawberries. The condition of the exposed honey bee colonies as assessed by colony status, development of the brood as well as strength and size of the brood nest, was not affected by the treatment. No evidence of disturbance or termination of the brood development caused by the exposure to the treated crop was seen in the exposed colonies. Moreover, adverse effects on honey bees, as determined by mortality and flight intensity as well as adverse effects on honey bee behaviour have not been observed.

Overall, it can be concluded from the available field studies in target crops - conducted under realistic-worst case use conditions - that spirotetratmat can be applied via foliar application to flowering citrus, flowering vegetables and flowering strawberries without adverse effects on honey bee brood development, honey bee colony performance, storage behaviour of exposed honey bee colonies, mortality, foraging activity, behaviour, colony vitality and colony survival. It can be further concluded that foliar applications to flowering citrus, flowering vegetables and flowering strawberries do not interfere with bee keeping activities under typical commercial use conditions.

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Crop-specific risk assessment, considering the envisaged use pattern

Use of spirotetramat in citrus (BBCH 71 - 78)

The envisaged use of spirotetramat in citrus groves accounts for post-flowering application rates of up to and including 288 g a.s./ha. As a consequence of the post-flowering application of spirotetramat, exposure of honey bees can be expected to be limited. Moreover, in total three independent field studies have been conducted, accounting for one or two sequential spirotetramat applications during flowering within medium-sized to large scale citrus plantations and application rates of up to and including 96 g a.s./ha/m canopy hight, which resulted in effective ha-based application rates of up to and including ≈ 280 g a.s./ha. In none of the three field studies, where spirotetramat has been applied during the citrus flowering period, adverse effects on honey bee brood development, honey bee colony performance and well as on the storage behaviour of honey bee colonies have been observed. Moreover, in none of the three citrus-studies, adverse effects on honey bee mortality, foraging activity and honey bee behaviour have been detected.
Overall, it can be concluded that the envisaged, specified post-flowering use of spirotetramat in citrus plantations does not pose an unacceptable risk to honey bees, including bee brood.

Use of spirotetramat in lettuce (BBCH 42 - 43)

The envisaged use of spirotetramat in lettuce accounts for application rates of up to and including 72 g a.s./ha, during the development of harvestable, vegetative plant parts. Lettuce, however, is not flowering in commercial cultivation and as such, honey bees are not attracted by the crop. Moreover, spray applications of spirotetramat during flowering are excluded by the envisaged application window.
Overall, it can be concluded that the envisaged use of spirotetramat in lettuce does not pose an unacceptable risk to honey bees, including bee brood.

IIIA1 10.4.1.2 Contact exposure Q_{HC}

The risk assessment for contact exposure has been conducted together with the one for oral exposure and is presented at Point IIIA1 10.4.1.1.

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IIIA1 10.4.2 Acute toxicity of the preparation to bees

Report:	**KIIIA1 10.4.2/01, [redacted]; 2005**
Title:	Acute toxicity of BYI 08330 150 OD to the honeybee *Apis mellifera* L. under laboratory conditions. Date: 2005-10-18
Organisation:	[redacted], Germany Bayer CropScience GmbH, [redacted], Germany
Report No.:	05 10 48 032; M-259122-01-2
Publication:	unpublished
Dates of experimental work:	June 21, 2005 - June 23, 2005
Guidelines:	OECD 213: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Oral Toxicity Test, (adopted September 1998) OECD 214: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Contact Toxicity Test, (adopted September 1998)
Deviations:	none
GLP	yes (certified laboratory)

Executive summary

The aim of the study was to determine acute oral and contact toxicity of BYI 08330 OD 150 to Honey bees (*Apis mellifera* L.). 3 replicates, each consisting of 10 bees in one cage per concentration were exposed to test concentrations of 200.0, 100.0, 50.0, 25.0 and 12.5 µg a.s./bee in the contact test and 198.9, 99.9, 49.9, 25.0 and 12.5 µg a.s./bee in the oral test. Endpoints were mortality and behaviour of the bees compared to control up to 48 h after application. Perfection EC 400 was used as toxic reference. The calculated LD_{50} (48 h) was 1072.9 µg product per bee (equivalent to 162.0 µg a.s./bee) in the contact toxicity test and 607.1 µg consumed product per bee (equivalent to 91.7 µg consumed a.s./bee) in the oral toxicity test.

Material and Methods

A Materials

1. Test material	Spirotetramat (BYI 08330) OD 150
Description	Light brown suspension
Lot/batch No.	Batch no.: 08030/0189(0152), TOX no.: 07034-00
Analytical content of a.s.	148.89 g/L
Stability of test compound	Expiry date: 2006-03-10, when stored at room temperature
2. Vehicle and/or positive control	Tween® 80 (0.1% v/v) Perfekthion EC 400 (analysed content: Dimethoate: 408.7 g/L)
3. Test animals	
Species	Honey bee (*Apis mellifera* L.), worker bees
Age	approx. 2-4 weeks
Source	Healthy, disease free and queen-right bee colonies obtained from [redacted], Germany
Acclimation period	Approx. 1-2 hours (corresponding to the starvation period in the oral toxicity test)
Environmental conditions	
Temperature	25 – 26°C (according to study plan: (25 ± 2)°C)
Relative humidity	59 - 61% (according to study plan: around 50-70%)

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Photoperiod	Constant darkness throughout the test (diffuse artificial light of about 100 lx only during handling and assessments)
Ventilation	By the air-conditioning equipment of the climatic chamber

B Study design and methods

1. In life dates — June 21, 2005 -June 23, 2005
2. Experimental treatments

Test units were disposable cages of cardboard with holes in the bottom for ventilation and a glass plate in front for observation of the bees (dimensions inside: 80 mm x 45 mm x 65 mm). 10 bees were used per test unit, 3 replicates per test item dose level, controls and toxic standard dosages (i.e. 30 individuals per treatment group). Food was 50% (w/v) aqueous sucrose solution, feeding was continuously during the test.

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

Contact toxicity Test item [µg product/bee]	 BYI 08330 [µg a.s./bee]	Oral toxicity* Test item [µg product/bee]	 BYI 08330 [µg a.s./bee]
1324.5	200.0	1317.0	198.9
662.2	100.0	661.3	99.9
331.1	50.0	330.7	49.9
165.6	25.0	165.6	25.0
82.8	12.5	82.7	12.5

*based on actual intake

Applied/exposed volume in the contact test was 4 µL/bee (test item is miscible 0.1% v/v Tween solution). Because of the low content of the active substance in the test item formulation it was necessary to increase the application volume of the test item solution up to 4 µL/bee to meet the requirement of a maximum applied dose of 200 µg a.s./bee.
Applied/exposed volume in the oral toxicity test was 200 µL sucrose solution/10 bees = 20 µL/bee.

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

Contact toxicity		Oral toxicity	
Reference item [µg product/bee]	Dimethoate [µg a.s./bee]	Reference item [µg product/bee]	Dimethoate [µg a.s./bee]
1.315	0.500	1.302	0.495
0.657	0.250	0.655	0.249
0.329	0.125	0.329	0.125
0.164	0.0625	0.164	0.0625

Applied/exposed volume in the contact test was 2 µL/bee.
Applied/exposed volume in the oral toxicity test was 200 µL sucrose solution/10 bees = 20 µL/bee

3. Observations

The number of dead and affected bees was counted at 4, 24 and 48 hours. During assessments times any behavioural abnormalities of the bees were recorded, considering the following parameters compared to control: healthy or affected (paralysis, lateral position,

lying on the back), any differences in activity, in position within the cage (all on the bottom), abnormal amount and colour of excretion.
For statistical calculation of the mortality results the Fisher's Exact Binomial test was used. The accepted significance level was $p \leq 0.05$ (one-sided greater). The median lethal dose (LD_{50}) along with the 95% confidence limits was calculated by Probit analysis according to the maximum likelihood method (FINNEY 1971) or according to the linear weighed regression. The goodness-of-fit of the model was evaluated by Pearson's X^2 test. The calculation of statistical significance and the LD_{50} was performed with the computer programs ToxRat Professional 2.09.

Results and Discussion

A. Findings

The validity criteria were met as mortality in the control was ≤ 10% (being 0% in the contact and oral toxicity tests after 48 hours) and the LD_{50} 24 h values for the toxic standard were in the postulated range of 0.1 - 0.30 µg a.s./bee (contact) and 0.1 - 0.35 µg a.s./bee (oral) (being 0.263 µg a.s./bee and 0.194 µg a.s./bee in the contact and the oral toxicity tests, respectively).

Oral and contact toxicity LD_{50} values of bees treated with BYI 08330 150 OD

Test item	BYI 08330 150 OD				
Test object	Honeybee *Apis mellifera L.*				
Exposure	contact, oral				
	treatment time	contact toxicity test		oral toxicity test	
		µg product/bee	µg a.s./bee	µg product/bee	µg a.s./bee
Test item BYI 08330 150 OD	24 h 95%-cl lower upper	1151.0 933.8 1418.8	-	607.1 506.7 727.4	-
	48 h 95%-cl lower upper	1072.9 894.3 1287.3	-	607.1 506.7 727.4	-
Reference item Dimethoate EC 400	24 h 95%-cl lower upper	-	0.263 0.225 0.308	-	0.194 0.171 0.220
	48 h 95%-cl lower upper	-	0.243 0.209 0.283	-	0.189 0.166 0.214

cl: confidence limits

In the contact toxicity test no statistically significant effects of the test item BYI 08330 150 OD on survival were observed at tested doses of 82.8, 165.6, 331.1 and 662.2 µg product per bee (0, 0, 3.3 and 10% mortality, respectively) during 48 hours. For the tested dose of 1324.5 µg product per bee statistically significant effects of the test item on survival were observed (70% mortality) during 48 hours. The calculated LD_{50} (48 h) was 1072.9 µg product per bee (equivalent to 162.0 µg a.s./bee) in the contact toxicity test.
In the oral toxicity test no statistically significant effects of the test item on survival were observed at consumed doses of 82.7 and 165.6 µg product per bee (0 and 0% mortality, respectively) during 48 hours. For the consumed doses of 330.7, 661.3 and 1317.0 µg product per bee statistically significant effects of the test item on survival were observed (23.3, 50.0 and 90.0% mortality, respectively) during 48 hours. The calculated LD_{50} (48 h) was 607.1 µg consumed product per bee (equivalent to 91.7 µg consumed a.s./bee) in the oral toxicity test.

B. Observations

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In the contact toxicity test no effects on behaviour were observed in honeybees after exposure to doses of 82.8, 165.6 and 331.1 µg product per bee. Bees exposed to doses equal or greater than 662.2 µg product per bee were affected at the 4 and 24 h assessments. During assessments conducted 48 hours after contact exposure bees had generally recovered and no different behaviour for all surviving bees exposed up to a dose of 1324.5 µg product/bee compared to control bees was observed.
In the oral toxicity test no effects on behaviour were observed in honeybees consuming doses equal or less than 330.7 µg product/bee at the 4 hour assessment. After consuming doses of 661.3 and 1317.0 µg product/bee most bees were affected. During assessments conducted 24 hours after oral exposure all surviving bees consuming doses equal or greater than 661.3 µg product/ bee were still affected or immobile. At the assessment conducted 48 hours after oral exposure bees had generally recovered and no different behaviour for all surviving bees consuming doses up to 1317.0 µg product/bee compared to control bees was observed.

In the reference treatments apathy, discoordinated movements and immobility were observed before bees died.

Conclusion

The calculated LD_{50} (48 h) was 1072.9 µg product per bee (equivalent to 162.0 µg a.s./bee) in the contact toxicity test and 607.1 µg consumed product per bee (equivalent to 91.7 µg consumed a.s./bee) in the oral toxicity test.

([redacted] M., 2005)

Report:	**KIIIA1 10.4.2/02, [redacted] S. & [redacted] T., 2008**
Title:	Effects of Spirotetramat SC 100 G (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory Date: 2008-02-07
Organisation:	[redacted], Germany Bayer CropScience AG, [redacted], Germany
Report No.:	34251035; M-298419-01-1
Publication:	Unpublished
Dates of experimental work:	2007-08-28 to 2007-08-31
Guidelines:	OECD 213 and 214 (1998)
Deviations:	None
GLP:	Yes (certified laboratory)

Executive summary

The aim of the study was to determine acute oral and contact toxicity of Spirotetramat SC 100 G to Honey bees (*Apis mellifera* L.) in a laboratory limit test.

Apis mellifera (50 worker bees per dose) were exposed for 48 hours to a single dose of 109.0 µg a.s. per bee for feeding (oral value based on the actual intake of the test item) and for topical application (contact) with a single dose of 100.0 µg a.s. per bee. 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. The toxicity of Spirotetramat SC 100 G was tested in both an acute contact and an oral toxicity test on honey bees.

The LD_{50} (48 h) was > 100.0 µg a.s./bee in the contact toxicity test.
The LD_{50} (48 h) was > 109.0 µg a.s./bee in the oral toxicity test.

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MATERIAL AND METHODS

A Materials

1. Test material	**Spirotetramat SC 100 G**
Description	liquid, white
Lot/batch No.	Specification: Batch ID.: 2007-005473 TOX No.: 07986-00
Analytical content a.s.	101 g/L, (9.35% w/w)
Stability of test compound	Test item is considered stable under test conditions. Expiry date: 2008-06-11, when stored in original container at +2 °C to +30 °C, in the dark
2. Vehicle and/or positive control	Vehicle: oral test: 50% aqueous sugar solution (in tap water); contact test: tap water with 0.5% Adhäsit (batch No.: 0130818, active ingredient content: 100 g/L Marlopon (nominal) - improves spreading of the test droplet on the water-repellent hairs on the thorax of bees) (applied after anesthetization). Positive control: Perfekthion EC 400 (batch No.: 0130818: 1812, analysed content: Dimethoate: 414.4 g/L)
3. Test animals	
Species	Honey bee (*Apis mellifera* L.)
Age	female adult worker bees
Source	Honey bee colonies, disease-free and queen-right, bred by [redacted]
Collection	Collected in the morning of use.
Environmental conditions	Incubators
Temperature	25°C
Relative humidity	35 - 53%
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 August 28, 2007 - August 31, 2007

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).

Control: Contact test: tap water + Adhäsit treated control (applied after anesthetization with CO_2); Oral test: 50% aqueous sugar solution (in tap water).

Test item: Nominal dosage of the test item in the contact and in the oral test was 100 µg a.s./bee.

Toxic reference item: 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).

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 Spirotetramat SC 100 G in an appropriate carrier (tap water + 0.5% Adhäsit) was placed on the dorsal bee thorax using a Burkard – Applicator.

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For the control one 5 µL droplet of tap water containing 0.5% Adhäsit was used. The reference item was also applied in 5 µL tap water (dimethoate made up in tap water containing 0.5% Adhäsit).

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; [redacted] 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:

Aqueous stock solutions of the test item and reference item were prepared in such a way that they had the respective target concentration of the test item once they were subsequently mixed with sugar syrup at a ratio of 1 : 1. After mixing of these test solutions with ready-to-use sugar syrup (composition of the sugar component: 30% Saccharose, 31% Glucose, 39% Fructose) the final concentration of sugar syrup in the test item solutions offered to the bees was 50%. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 45 minutes for the test item treatments). After a maximum of 45 minutes, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

The target dose levels (*e.g.* 100 µg a.s./bee nominal) would have been obtained if 20 mg/bee of the treated food was ingested. In practice, higher (or lower) dose levels were obtained as the bees had a higher or lower uptake of the test solutions than the nominal 20 mg/bee.

3. Observations

The number of dead bees was determined 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 reference item were estimated according to moving average computations ([redacted] and Weil, 1952).

The software used to perform the statistical analysis was ToxRat Professional, Version 2.09, ® ToxRat Solutions GmbH, © 2005.

Results and Discussion

A. Findings

The validity criteria were met as mortality in the 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 below.

Mortality and behavioural abnormalities of the bees in the contact toxicity test (results are average from 5 replicates (ten bees each) per dosage/control)

Dosage [µg a.s./bee]	after 4 hours		after 24 hours		after 48 hours	
	mortality	behav. abnorm.	mortality	behav. abnorm.	mortality	behav. abnorm.
	mean [%]	mean [%]	mean [%]	mean [%]	mean [%]	mean [%]
Test item						
100	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						
0.30	0.0	12.0	76.0	2.0	80.0	4.0
0.20	0.0	0.0	18.0	4.0	32.0	16.0
0.15	0.0	0.0	0.0	0.0	8.0	4.0
0.10	0.0	0.0	2.0	0.0	2.0	0.0

behav. abnorm. = behavioural abnormalities water = CO_2/water treated control

In the oral toxicity test the maximum nominal test level of Spirotetramat SC 100 G (100 µg a.s./bee) corresponded to an actual intake of 109 µg a.s./bee. At this concentration level no mortality occurred within 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)

Dosage [µg a.i./bee]	after 4 hours		after 24 hours		after 48 hours	
	mortality	behav. abnorm.	mortality	behav. abnorm.	mortality	behav. abnorm.
	mean [%]	mean [%]	mean [%]	mean [%]	mean [%]	mean [%]
Test item						
109	0.0	0.0	0.0	0.0	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Reference item						
0.30	24.0	42.0	92.0	8.0	100.0	0.0
0.16	0.0	0.0	64.0	6.0	74.0	0.0
0.09	0.0	0.0	8.0	6.0	12.0	0.0
0.06	0.0	0.0	4.0	0.0	4.0	0.0

behav. abnorm. = behavioural abnormalities water = CO_2/water treated control

Toxicity to Honey Bees; laboratory tests

Test Item	Spirotetramat SC 100 G	
Test object	*Apis mellifera*	
Application rate µg a.s./bee	109.0	100.0
Exposure	oral (sugar solution)	contact (solution in Adhäsit (0.5%)/water)
LD_{50} µg a.s./bee	> 109.0	> 100.0

B. Observations

At the end of the contact toxicity test (48 hours after application), there was 0.0% mortality at 100.0 µg a.s./bee. No mortality occurred in the control (water + 0.5% Adhäsit).
In the oral toxicity test the maximum nominal test level of Spirotetramat SC 100 G (100 µg a.s./bee) corresponded to an actual intake of 109.0 µg a.s./bee. This dose level led to no mortality after 48 hours.
No mortality occurred in the control (50% sugar solution).
No test item induced behavioural effects were observed at any time.

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CONCLUSION

The toxicity of Spirotetramat SC 100 G was tested in both an acute contact and an oral toxicity test on honey bees. The LD_{50} (48 h) was > 100.0 µg a.s./bee in the contact toxicity test. The LD_{50} (48 h) was > 109.0 µg a.s./bee in the oral toxicity test.

([redacted] S. & [redacted] T., 2008)

Report:	**KIIA1 10.4.2/03, [redacted] S.; 2004**
Title:	Effects of BYI 08330 100 OD (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory Date: 2004-09-30
Organisation:	[redacted], Germany Bayer CropScience GmbH, [redacted], Germany
Report No:	20941035; M-092624-01-1
Publication:	unpublished
Dates of experimental work:	August 17, 2004 – August 27, 2004
Guidelines:	OECD 213 and 214 (1998); Recommendations of the ICPBR group, 1999
Deviations:	none
GLP:	yes (certified laboratory)

Executive summary

The aim of the study was to determine acute contact and acute oral toxicity of Spirotetramat OD 100 to honey bees (*Apis mellifera* L.) in a laboratory toxicity test.

The toxicity of Spirotetramat OD 100 was tested in both an acute contact and an acute oral toxicity test on honey bees. *Apis mellifera* (30 worker bees per dose/ control) were exposed to the test item for 96 hours in the contact test and for 48 hours in the oral test. Test concentrations were 200, 150, 100, 50 and 25 µg a.s. per bee for topical application in the contact test and 114.7, 105.0, 55.8, 26.9 and 13.6 µg a.s. per bee for feeding in the oral test. Mortality was assessed after 4, 24 and 48 hours (contact and oral test), and additionally after 72 and 96 hours (contact test) because of increasing mortality between 24 and 48 hours in the contact test. Dimethoate 400 g/L (nominal) was used as toxic reference.

In the contact toxicity test behavioural abnormalities attributed to exposure to the test item such as discoordinated movements and apathy were observed during the whole experimental time. In the oral toxicity test first behavioural abnormalities were observed during the 4 hours check in all treatment groups, except the 13.6 µg a.s. per bee group. No behavioural impairments were found in any dose group during the 48 hours check.

The LD_{50} (48 h + 96 h) was 98.7 and 74.3 µg a.s./bee in the contact toxicity test, respectively.
The LD_{50} (24 h + 48 h) was 53.2 and 57.7 µg a.s./bee in the oral toxicity test, respectively.

MATERIAL AND METHODS

A Materials

1. Test material	Spirotetramat OD 100
Description	liquid, brown
Lot/batch No.	08030/0110(0073) TOX06593-00
Analytical content a.s.	102.02 g/L
Stability of test compound	Test item is considered stable under test conditions. Expiry date: February 2005, when stored in original container at +5 °C to + 30 °C, in the dark

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2. Vehicle and/or positive control	Vehicle: oral test: tap water + syrup solution; contact test: tap water + 1% Adhäsit (batch No.: 0100208; active ingredient/content: 100 g/L Triethanolamin-Dodecylbenzolsulfonat (nominal) - improves spreading of the test droplet on the water-repellent hairs on the thorax of bees) (applied with anesthetization). Positive control: Perfekthion EC 400 (batch No.: 0100208: 1800, analysed content: Dimethoate: 396.1 g/L)
3. Test animals	
Species	Honey bees (*Apis mellifera* L.)
Age	4 – 6 week old female worker bees
Source	Honey bee colonies, disease-free and queen-right, bred by [redacted]
Collection	Collected in the morning of use.
Environmental conditions	Incubators
Temperature	25°C
Relative humidity	65 - 76%
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 — August 17, 2004 - August 27, 2004

2. Experimental treatments

Test units were stainless steel chambers of 10 cm x 8.5 cm x 5.5 cm (length x width x height), the front side was a removable glass sheet, the bottom was perforated with 98 ventilation holes (Ø 1 mm), the inner walls were lined with filter paper. 10 bees were used per test unit, 3 replicates per test item dose level, controls and toxic standard dosages (i.e. 30 individuals per treatment group). Food was commercial ready-to-use syrup (Apiinvert, 30% Saccharose, 31% Glucose, 39% Fructose).

Control: Contact test: CO_2/tap water + Adhäsit[10] treated control; Oral test: tap water/ syrup control.

Test item: Contact test: Nominal dosage of the test item was 200, 150, 100, 50 and 25 µg a.s./bee; Oral test: Nominal dosage of the test item was 200, 100, 50, 25 and 12.5 µg a.s./bee (measured dosage of the test item was 114.7, 105.0, 55.8, 26.9 and 13.6 µg a.s./bee).

Toxic reference item: 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). Measured dosage of the toxic reference was 0.34, 0.17, 0.08 and 0.04 µg dimethoate/bee (oral test).

Application of the test item in the contact test:

Bees were anaesthetized with CO_2 in the contact test. One single 5 µL droplet of BYI 08330 100OD in solvent (solvent = water + 1 % Adhäsit) was placed on the ventral bee thorax using a Burkard Applicator. For the controls one 5 µL droplet of tap water with 1 % Adhasit was used. The toxic standard was applied in 5 µL tap water with 1% Adhäsit (a 5 µL droplet was chosen in deviation to the guideline recommendation of 1 µL, since a higher volume ensured a more reliable dispersion of the test item; [redacted] experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected)

Application of the test item in the oral test:

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

BYI 08330 100 OD dilutions in tap water were prepared in order to receive a final 50% syrup (sugar) solution, if these dilutions were mixed with syrup in a relation 1+1. For the control, tap water was mixed with syrup as well to receive a 50% sugar solution. This diet was offered in syringes which were weighed before and after introduction into the cages (after 6 hours the test item treated food was replaced by fresh, untreated food) [The desired dosages (e.g. 100 µg a.s./bee) should be obtained, when 20 mg/bee of the treated food would be ingested. Higher (or even lower) doses were obtained due to an increased (or decreased) food uptake by the bees].

3. Observations
The number of dead bees was determined after 4 hours (first day), 24 and 48 hours (contact and oral test), and additionally after 72 and 96 hours (contact test). Behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4 hours (first day); 24 and 48 hours (contact and oral test); additionally after 72 and 96 hours (contact test).
Results obtained from the bees treated with test item were compared to those obtained from the toxic standard and the controls.
The contact and oral LD_{50} of the test item and the toxic standard were estimated with Probit Analysis (according to Finney 1971). The 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, ® ToxRat Solutions GmbH, ©2001-2004.

RESULTS AND DISCUSSION

A. Findings

The validity criteria were met as mortality in the 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 below.

Contact test:

Mortality and behavioural abnormalities of the bees in the contact toxicity test (results are average from 3 replicates (ten bees each) per dosage/control)

Dosage [µg a.s./bee]	after 24 hours		after 48 hours		after 72 hours		after 96 hours	
	mortality mean [%]	behav. abnorm. mean [%]	mortality mean [%]	behav. abnorm. mean [%]	mortality mean [%]	behav. abnorm. mean [%]	mortality mean [%]	behav. abnorm. mean [%]
Test item								
200.0	53.3	46.7	90.0	10.0	96.7	3.3	100.0	0.0
150.0	20.0	80.0	66.7	26.7	80.0	13.3	90.0	3.3
100.0	30.0	20.0	63.3	6.7	66.7	0.0	66.7	0.0
50.0	0.0	3.3	0.0	13.3	10.0	3.3	10.0	3.3
25.0	0.0	10.0	6.7	16.7	16.7	0.0	23.3	0.0
Water control	0.0	0.0	0.0	0.0	3.3	0.0	3.3	0.0
Toxic standard								
0.30	96.7	0.0	100.0	0.0	100.0	0.0	100.0	0.0
0.20	86.7	3.3	93.3	3.3	96.7	0.0	96.7	0.0
0.15	63.3	16.7	90.0	3.3	96.7	0.0	96.7	0.0
0.10	6.7	0.0	10.0	3.3	26.7	6.7	40.0	0.0

behav. abnorm. = behavioural abnormalities
water = CO_2/water treated control

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Oral test:

Mortality and behavioural abnormalities of the bees in the oral toxicity test (results are average from 5 replicates (ten bees each) per dosage/control)

Dosage * [µg a.s./bee]	after 4 hours mortality mean [%]	after 4 hours behav. abnorm. mean [%]	after 24 hours mortality mean [%]	after 24 hours behav. abnorm. mean [%]	after 48 hours mortality mean [%]	after 48 hours behav. abnorm. mean [%]
Test item						
114.7	33.3	66.7	96.7	3.3	100.0	0.0
105.0	23.3	70.0	66.7	10.0	76.7	0.0
55.8	6.7	40.0	36.7	0.0	36.7	0.0
26.9	16.7	3.3	26.7	3.3	30.0	0.0
13.6	0.0	0.0	10.0	0.0	10.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Toxic standard						
0.30	6.7	33.3	86.7	3.3	93.3	0.0
0.17	0.0	23.3	60.0	10.0	70.0	0.0
0.08	0.0	6.7	20.0	6.7	36.7	0.0
0.04	0.0	0.0	0.0	0.0	0.0	0.0

behav. abnorm. = behavioural abnormalities water = CO_2 water treated control
* measured test concentrations

Toxicity to Honey Bees; laboratory tests

Test Item	Spirotetramat OD 100	
Test object	*Apis mellifera*	
Application rate µg a.s./bee	114.7	200.0
	105.0	150.0
	55.8	100.0
	26.9	50.0
	13.6	25.0
Exposure	oral (sugar solution)	contact (solution in Adhäsit (1%)/water)
LD_{50} µg a.s./bee [24 h]	53.2	201.5
LD_{50} µg a.s./bee [48 h]	57.7	98.7
LD_{50} µg a.s./bee [72 h]	--	84.4
LD_{50} µg a.s./bee [96 h]	--	74.3

B. Observations

The contact test was prolonged for further 48 hours up to 96 hours because of increasing mortality between 24 and 48 hours. Mortality occurred in all groups dosed with BYI 08330 100 OD, more or less increasing with dose levels. 3.3% control mortality occurred at test end (96 hours). During the whole experimental time behavioural impairments such as discoordinated movements and apathy were observed in the contact test.

Oral doses of 114.7, 105.0, 55.8, 26.9 and 13.6 µg a.s./bee led to dose dependent mortality levels ranging from 100.0 to 10.0% at test end. No control mortality occurred. The highest nominal test rate of 200 µg a.s./bee could not be achieved, since the bees died or were behaviourally impaired and therefore were not able to ingest the desired volume of contaminated food.

In the oral test during the first 4 hours discoordinated movements and/or apathy were observed in all dose groups except in the 13.6 µg a.s./bee group. During the 24 hours check a few bees had moving coordination problems but this was not dose related. No behavioural impairments were found in any dose group during the 48 hours check.

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CONCLUSION

The toxicity of Spirotetramat OD 100 was tested in both an acute contact and an acute oral toxicity test on honey bees. The following LD_{50} values were found for both tests:

Mortality:	Contact LD_{50} (24h) of BYI08330 100 OD: 201.5 µg a.s./bee
	Contact LD_{50} (48h) of BYI 08330 100 OD: 98.7 µg a.s./bee
	Contact LD_{50} (72h) of BYI 08330 100 OD: 84.4 µg a.s./bee
	Contact LD_{50} (96h) of BYI 08330 100 OD: 74.3 µg a.s./bee
	Oral LD_{50} (24h) of BYI 08330 100 OD: 53.2 µg a.s./bee
	Oral LD_{50} (48h) of BYI 08330 100 OD: 57.7 µg a.s./bee

During the whole experimental time behavioural impairments such as discoordinated movements and apathy were observed in the contact test. In the oral test, behavioural abnormalities occurred initially after 4 hours of the test; however, no abnormalities were found in any of the dose groups after 48 hours.

([redacted] S., 2004)

Report:	**KIIIA1 10.4.2/04, [redacted]; 2005**
Title:	Effects of BYI08330 240 SC (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory. Date: 2005-02-01
Organisation:	[redacted], Germany Bayer CropScience GmbH, [redacted], Germany
Report No.:	21371035; M-245084-01-1
Publication:	unpublished
Dates of experimental work:	August 17, 2005 - August 20, 2005
Guidelines:	OECD 213, Honeybees, Acute Oral Toxicity Test (1998) OECD 214, Honeybees, Acute Contact Toxicity Test (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 240 SC to honey bees (*Apis mellifera* L.) in a laboratory limit test. 5 replicates, each consisting of 10 bees in one cage per test concentration were exposed to test concentrations of 100 µg a.s./bee in the contact test and 106.3 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 no mortality occurred in the 100.0 µg a.s./bee group, the contact LD_{50} must be considered as clearly in excess of 100.0 µg a.s./bee. Since no mortality occurred in the 106.3 µg a.s./bee group, the contact LD_{50} must be considered as clearly in excess of 106.3 µg a.s./bee

MATERIAL AND METHODS

A Materials

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1. Test material	BYI 08330 240 SC
Description	liquid, white
Lot/batch No.	Batch no.: 07218/0019(0019) Tox no.: 06753-00
Analytical content a.s.	236.5 g/L
Stability of test compound	Test item is considered stable under test conditions. Expiry date: 2005-06-29, when stored in original container at room temperature, in the dark
2. Adjuvant	RME EW 500 (rape methyl ester)
Description	liquid, white
Lot/batch No.	Batch no.: 05778/0209(0128) Tox no.: 06752-00
Analytical content a.s.	not indicated
Stability	Adjuvant is considered stable under test conditions. Expiry date: 2004-12-29, when stored in original container at room temperature, in the dark
3. Vehicle and/or positive control	Water Perfekthion EC 400 (analysed content: Dimethoate: 396.1 g/L)
4. 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 [redacted]
Collection	Collected in the morning of use.
Environmental conditions	Incubators
Temperature	25°C
Relative humidity	75 - 79%
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: August 17, 2004 – August 20, 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 treated control was used; in the oral test a tap water/sugar control.

<u>Application of the test item in the contact test:</u>

Bees were anaesthetized with CO2 in the contact test. One single 5 µL droplet of BYI08330 240 SC in solvent (solvent = water) was placed on the ventral bee thorax using a Burkard-Applicator. For the controls one 5 µL droplet of tap water with 1% Adhäsit was used. The toxic standard was applied in 5µL water with 1% Adhäsit (a 5 µL droplet was chosen in

deviation to the guideline recommendation of 1 µL, since a higher volume ensured a more reliable dispersion of the test item; [redacted] 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:

BYI 08330 240 SC dilutions in tap water were prepared in order to receive a final 50% syrup (sugar) solution. These dilutions were mixed with syrup in a relation 1+1. For the controls tap water was mixed with syrup as well to receive a 50% sugar solution. This diet was offered in syringes which were weighed before and after introduction into the cages (after one hour the test item treated food was completely ingested by the bees and 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} (24 h and 48 h) of the toxic standard was estimated with Probit Analysis (according to Finney 1971). 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 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).

Dosage [µg a.s./bee]	after 4 hours mortality mean [%]	after 4 hours behav. abnorm. mean [%]	after 24 hours mortality mean [%]	after 24 hours behav. abnorm. mean [%]	after 48 hours mortality mean [%]	after 48 hours behav. abnorm. mean [%]
Test item						
100	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
Toxic standard						
0.30	16.0	18.0	98.0	0.0	98.0	0.0
0.20	2.0	4.0	86.0	8.0	100.0	0.0
0.15	0.0	0.0	26.0	18.0	66.0	4.0
0.10	0.0	0.0	0.0	2.0	4.0	0.0

behav. abnorm. = behavioural abnormalities   water = CO_2/water treated control

In the oral toxicity test the maximum nominal test level of BYI 08330 240 SC (100 µg a.s./bee) corresponded to an actual intake of 106.3 µg a.s./bee. At this concentration level no mortality occurred within 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)

Dosage [µg a.s./bee]	after 4 hours mortality mean [%]	after 4 hours behav. abnorm. mean [%]	after 24 hours mortality mean [%]	after 24 hours behav. abnorm. mean [%]	after 48 hours mortality mean [%]	after 48 hours behav. abnorm. mean [%]
Test item 106.3	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
Toxic standard 0.33	10.0	48.0	80.0	8.0	90.0	2.0
0.16	2.0	2.0	42.0	8.0	56.0	2.0
0.08	0.0	0.0	4.0	0.0	18.0	0.0
0.04	0.0	0.0	0.0	0.0	0.0	0.0

behav. abnorm. = behavioural abnormalities water = CO_2/water treated control

Oral and contact toxicity LD_{50} values of bees treated with BYI 08330 240 SC

	LD_{50} [µg a.s./bee] Contact test	LD_{50} [µg a.s./bee] Oral test
Test item (48 h)	> 100.0	> 106.3
Toxic standard (24 h) (95% Confidence limit)	0.17 (0.16 - 0.18)	0.19 (0.17 - 0.22)

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 240 SC 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 > 106.3 µg a.s./bee in the oral toxicity test.

([redacted] S.; 2005)

IIIA1 10.4.2.1 Acute oral toxicity

Studies with the preparation on acute oral toxicity are summarised in IIIA1 10.4.2.

IIIA1 10.4.2.2 Acute contact toxicity

Studies with the preparation on acute contact toxicity are summarised in IIIA1 10.4.2.

IIIA1 10.4.3 Effects on bees of residues on crops

Covered and performed in field studies, see Annexpoints IIIA1 10.4.5 and 10.4.6.

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IIIA1 10.4.4 Cage tests

Due to the findings presented above, no further studies are required. The Q_{HO} and Q_{HC} values are < 50.

Report:	**KIIIA1 10.4.4/01, [redacted]; 2007**
Title:	Summary of orientating research tunnel trials on effects of spirotetramat to honey bees according to EPPO 170. Date: 2007-10-17
Organisation:	[redacted], Germany Bayer CropScience AG, [redacted], Germany
Report No.:	PA07-174; M-294216-01-1
Publication:	unpublished
Dates of experimental work:	not applicable
Guidelines:	none
Deviations:	not applicable
GLP:	not applicable

For research and product positioning purposes Bayer CropScience AG performed orientating honey bee semi-field (tunnel) studies with Spirotetramat. The objectives of these studies were to investigate potential side effects of different formulations and rates of spirotetramat, and different pre-application intervals, to honey bees in flowering crops under tunnel conditions.
A total of nine honey bee tunnel studies were performed between 2002 and 2007 and will be summarised in this report. Seven of the reported studies were performed in Germany, two in Spain. For all studies *Phacelia tanacetifolia* was used as flowering crop with the exception of one study performed in 2007 on strawberry. All studies were carried out following the guidance of the EPPO 170 guideline under non-GLP conditions but according to GEP (good experimental practice).
The general test performance was as follows: Honeybee colonies of an appropriate size (approx. 3,000 – 3,500 honey bees) were confined in tunnels (44 m² and 60 m² in Spain and 50 m² (100 m² for the strawberry trial, due to lower density of the flowering crop) in Germany) with a flowering crop on a field. For the reported studies one or two replicates were set up for each treatment group. Each study comprised one untreated control, one toxic reference (fenoxycarb) and one to six different test item treatment groups. Honey bees were introduced into the tunnels some days prior to application to get familiar with the new environmental conditions (unless pre-flowering applications were tested). The bee colonies were examined for potential test item related effects for at least 7 days after the application inside the tunnels and for at least additional 14 days after removal from the tunnels. In particular, the endpoints adult, larval and pupal mortality, flight intensity and brood development were assessed, furthermore honey and pollen storage, egg laying and breeding activity, colony strength, hive weight and behavioural anomalies of the honey bees were recorded. The obtained results of each endpoint and treatment group from the post-application period were compared to those of the pre-application phase. The post-application results of the test item treatment rate(s) were also compared one with each other as well as to the control and the toxic reference. In this summary report, only the results of the endpoints related to bee brood development with regard to the larval development and mortality of pupae (where observed) are reported. All other brood (e.g. egg and capped cells, storage area) as well as all non-brood related endpoints (e.g. adult mortality, flight density, behavioural abnormalities, hive weight) were not impacted and therefore will not be reported in this summary, which is focussing on the potential of brood effects.
Due to the fact that only one or two replicates per treatment group were used in the reported studies, a statistical evaluation was not feasible. Hence, the determination of effects was performed on the basis of expert judgment.

Summary of Effects of Spirotetramat to Honey Bee Brood (Larvae)

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Trial ID	Country	Treatment	Formulation Type and content	Crop	Effect	Unclear effect	Recovery until
IY02DVG 0101002	Germany/2002	Spirotetramat 36 g a.i./ha RME 500 EW 0.2% a.i./v	SC 240 g a.i./L	Phacelia	3 DAA	-	7 DAA
		Spirotetramat 72 g a.i./ha RME 500 EW 0.2% a.i./v			3 DAA	-	7 DAA
		Spirotetramat 144 g a.i./ha RME 500 EW 0.2% a.i./v			3 DAA	-	7 DAA
		Spirotetramat 144 g a.i./ha			3 DAA	7 DAA	
IY02DVG 0101004	Germany/ 2002	Spirotetramat 36 g a.i./ha RME 500 EW 0.2% a.i./v	SC 240 g a.i./L	Phacelia	3 DAA – 7 DAA	15 DAA – 21 DAA	-
		Spirotetramat 72 g a.i./ha RME 500 EW 0.2% a.i./v			3 DAA	-	7 DAA
		Spirotetramat 144 g a.i./ha RME 500 EW 0.2% a.i./v			-	3 DAA	7 DAA
		Spirotetramat 144 g a.i./ha			-	-	-
IA03DVG 059G001	Germany/ 2002	Spirotetramat 144 g a.i./ha RME 500 EW 0.4% a.i./v	SC 240 g a.i./L OD 100 g a.i./L	Phacelia	4 DAA - 14 DAA	-	22 DAA
		Spirotetramat 144 g a.i./ha RME 500 EW 0.2% a.i./v			-	4 DAA – 7 DAA	14 DAA
		RME 500 EW 0.4% a.i./v			-	-	-
		Spirotetramat 140 g a.i./ha			7 DAA	4 DAA/ 14 DAA	22 DAA
IA03DVG 059G002	Germany/ 2003	Spirotetramat 144 g a.i./ha RME 500 EW 0.4% a.i./v	SC 240 g a.i./L OD 100 g a.i./L	Phacelia	4 DAA	7 DAA - 30 DAA	-
		Spirotetramat 144 g a.i./ha RME 500 EW 0.2% a.i./v			4 DAA – 7 DAA	15 DAA – 22 DAA	30 DAA
		RME 500 EW 0.4% a.i./v			-	-	-
		Spirotetramat 140 g a.i./ha			4 DAA	-	7 DAA
IA03VSS HS7JN01	Spain/ 2003	Spirotetramat 144 g a.i./ha RME 500 EW 0.1% a.i./v	SC 240 g a.i./L OD 100 g a.i./L	Phacelia	-	7 DAA	14 DAA
		Spirotetramat 140 g a.i./ha			-	7 DAA	14 DAA
		Spirotetramat 100 g a.i./ha			-	-	-
IA04DVG 053G001	Germany/ 2004	Spirotetramat 50 g a.i./ha	OD 150 g a.i./L	Phacelia	3 DAA – 8 DAA	15 DAA	21 DAA
		Spirotetramat 75 g a.i./ha			3 DAA – 8 DAA	15 DAA	21 DAA
		Spirotetramat 100 g a.i./ha			3 DAA – 15 DAA	-	21 DAA
		Spirotetramat 150 g a.i./ha			3 DAA – 8 DAA	15 DAA	21 DAA

Trial ID	Country	Treatment	Formulation Type and content	Crop	Effect	Unclear effect	Recovery until
IA04DVG 053G002	Germany/ 2004	Spirotetramat 50 g a.i./ha	OD 150 g a.i./L	Phacelia	3 DAA – 14 DAA	-[1]	-[1]
		Spirotetramat 75 g a.i./ha			3 DAA – 7 DAA	14 DAA	20 DAA
		Spirotetramat 100 g a.i./ha			3 DAA – 7 DAA	-	14 DAA
		Spirotetramat 150 g a.i./ha			3 DAA – 7 DAA	14 DAA	20 DAA
IA04DVG 065G001	Germany/ 2004	Spirotetramat 150 g a.i./ha 20 days prior to flowering	OD 150 g a.i./L	Phacelia	-	-	-
		Spirotetramat 150 g a.i./ha 15 days prior to flowering			-	-	-
		Spirotetramat 150 g a.i./ha 10 days prior to flowering			-	-	-
		Spirotetramat 150 g a.i./ha 5 days prior to flowering			4 DAE – 7 DAE	15 DAE	22 DAE
		Spirotetramat 150 g a.i./ha on flowering crop at bee flight			4 DAE – 7 DAE	15 DAE – 22 DAE	28 DAE
		Spirotetramat 150 g a.i./ha on flowering crop after bee flight			4 DAE - 7 DAE	-	15 DAE
IA04DVG 072G001	Germany/ 2004	Spirotetramat 150 g a.i./ha 20 days prior to flowering	OD 150 g a.i./L	Phacelia	-	7 DAE	15 DAE
		Spirotetramat 150 g a.i./ha 15 days prior to flowering			-	7 DAE	15 DAE
		Spirotetramat 150 g a.i./ha 10 days prior to flowering			-	-	-
		Spirotetramat 150 g a.i./ha 5 days prior to flowering			3 DAE – 7 DAE	-	15 DAE
		Spirotetramat 150 g a.i./ha on flowering crop at bee flight			3 DAE – 15 DAE	-	21 DAE
		Spirotetramat 150 g a.i./ha on flowering crop after bee flight			7 DAE	-	15 DAE
IA04VSS XW4JN 01	Spain/ 2004	Spirotetramat 75 g a.i./ha	OD 150 g a.i./L	Phacelia	7 DAA – 14 DAA	21 DAA- 31 DAA	-
		Spirotetramat 100 g a.i./ha			-	-	-
		Spirotetramat 150 g a.i./ha			-[2]	-[2]	-[2]
IA07DVG 014G001	Germany/ 2007	Spirotetramat 100 g a.i./ha	OD 150 g a.i./L	Straw-berry	-	9 DAA	16 DAA

[1] No egg laying activity after loss of the queen (not treatment related)
[2] bee in hive died presumably not due to treatment related effects
OD: oil dispersion
DAA: days after application

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DAE: days after beginning of exposure
-: no influence on bee brood observed

([redacted] U., 2007)

IIIA1 10.4.5 Field tests

Report:	**KIIIA1 10.4.5/01, [redacted]; 2006**
Title:	Assessment of Side Effects of Spirotetramat OD 150 on the Honey Bee (*Apis mellifera* L.) in the Field. Date: 2006-09-11
Organisation:	[redacted], Germany Bayer CropScience AG, [redacted], Germany
Report No.:	20061133/S1-BFEU; M-277194-02-2
Publication:	unpublished
Dates of experimental work:	May 01, 2006 -June 14, 2006
Guidelines:	OEPP/EPPO No. 170 (3) (2001)
Deviations:	no
GLP	yes (certified laboratory)

Executive summary

The objective of the study was to determine the effects of Spirotetramat OD 150 on the honey bee (*Apis mellifera* L.) in the field. Particular attention was directed to the development of the bee brood. The study was carried out in Spain (region: Valencia) on fields of flowering *Phacelia tanacetifolia*. In total there were three test fields, the test item treated field T1 (4 × 72 g a.s./ha, two times before flowering (no bees in the field) and two times during flowering of the crop and foraging activity of the bees), the test item field T2 (2 × 96 g a.s./ha during flowering of the crop and foraging activity of the bees) and the untreated control field using four bee colonies per field. Mortality, foraging activity of the bees, the condition of the colonies and the bee brood development was checked prior to and followed up after application.

It was concluded that neither test item treatments (T1 and T2) resulted in an adverse effect on honey bee as determined by mortality and flight intensity. Differences of bee behaviour between control and treatment groups were not observed. The condition of the colonies as assessed by colony strength and size of the brood nest was not affected by any of the treatments.

MATERIAL AND METHODS

A Materials

1. Test material	BYI 08330 OD 150
Description	Liquid, light brown
Lot/batch No.	Batch no.: 08030/0189(0152)
Content a.s.	15.1% (w/w) (analysed)
Stability of test compound	Approved until 2007-01-31 when stored at room temperature (25 ± 5°C)
2. Vehicle and/or positive control	Water
3. Test animals	
Species	Honey bee (*Apis mellifera* L.) Four normally developed, healthy and queen-right bee colonies were used per test field. Each colony contained one body with 12 frames and approx. 20000 bees per colony as typically used by beekeepers in Spain.

B Study design and methods

1. In life dates	May 01, 2006 -June 14, 2006

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2. Experimental treatments

The field test was located in Spain (region: Valencia). The crop *Phacelia tanacetifolia* was used in flowering stage. *P. tanacetifolia* is a crop specifically recommended in guideline OEPP/EPPO Guideline No. 170 (3) for field tests. In total there were three test fields, the test item treated field T1 (4 applications), the test item field T2 (2 applications) and the untreated control field. The field sizes were: test item field T1: 2025 m^2, test item field T2: 2016 m^2 and the control field C: 3306 m^2. The test fields were not close to other flowering crops or extensive blooming weeds, which may be attractive to bees and were separated by a distance of at least 2 km. In the test item field T1 Spirotetramat OD 150 was applied on the crop two times before flowering (no bees in the field) and two times during flowering of the crop and foraging activity of the bees. The applications were carried out in approximately 7-day intervals at an application rate each of 72 g a.s./ha in 200 L water/ha. In the test item field T2 the applications were carried out two times during flowering of the crop and foraging activity of the bees at an application rate each of 96 g a.s./ha in 200 L water/ha (7 days between the application dates). A field of untreated *P. tanacetifolia* was included as the control.

Commercial bee colonies were placed near the test fields 3 days before the 3rd application in the test item field T1 and before the 1st application in the test item field T2, respectively. To ensure that the bees are exposed to the test field detailed assessments of foraging activity were done before as well as after the application. Furthermore, exposure of the bees was assessed by visual inspection of the *Phacelia tanacetifolia* pollen on combs entered in the hives during the study.

3. Observations

Mortality and foraging activity of the bees were checked prior to first applications in the test fields during exposure (over 2 days and shortly before first application) and followed up after application (foraging activity: over 10 days after first application during exposure, mortality: over 28 days after first application during exposure. The condition of the colonies and the bee brood development were checked once before first application during exposure and five times afterwards (up to 26 days after first application during exposure).

The influence of the test item on the honey bees was evaluated by comparing the results of the test item treatments to those of the control treatment. The following points were assessed:

- Condition of the colonies (strength) and development of the bee brood
- Mortality in the field and in the bee traps in front of the hives
- Foraging activity (number of forager bees/m²/minute flowering crop)
- Behaviour of the bees on the crop and around the hive

Temperature and rainfall data were recorded at the a governmental weather station in Jalance, approximately 15 km (T1 field), 17 km (T2 field) and 18 km (control field) away from the field site. The humidity data were recorded at the a governmental weather station in [redacted], approximately 20 km (T1 and T2 field) and 25 km (control field) away from the field site. The degree of cloud coverage and wind speed during applications was assessed at the test fields.

No statistical evaluation

RESULTS AND DISCUSSION

A. Findings

Test item		Spirotetramat OD 150		
Test object		*Apis mellifera mellifera*		
Exposure		T1 and T2: spray treatment of Spirotetramat OD 150 during foraging activity at full flowering of the crop at two different application rates and number of applications		
Treatment group		**T1**	**T2**	**Control**
1st application date (01May06, before flowering): application rate g a.s./ha		72.0	-	-
2nd application date (08May06, before flowering): application rate g a.s./ha		72.0	-	-
3rd application date (17May06, during flowering/bee flight): application rate g a.s./ha		72.0	96.0	-
4th application date (24May06, during flowering/bee flight): application rate g a.s./ha		72.0	96.0	-
Spray volume pro ha [L water/ha]		200	200	-
Mean mortality [dead bees/colony/day]	Pre-appl. [DAA -2 to 0ba]:	43.4	135.4	87.7
	Pre-appl. [DAA 0ba]:	42.0	94.8	65.0
	Post-appl. [DAA 0aa]:	15.0	23.0	29.0
	Pre-appl. [DAA 7ba]:	22.0	17.8	2.8
	Post-appl. [DAA 7aa]:	3.8	6.8	2.3
	Post-appl. [DAA 0aa to 28]:	5.7	6.5	5.6
	Q_M:	0.1	<0.1	0.1
Mean number dead pupae/larvae per colony/day (post-application, DAA 0aa to 28)		0.3	0.1	<0.1
Mean flight intensity [foraging bees/minute/m²]	Pre-appl. [DAA -2 to 0ba]:	11.3	11.5	13.7
	Pre-appl. [DAA 0ba]:	15.0	13.0	12.4
	Post-appl. [DAA 0aa]:	11.9	14.4	14.4
	Pre-appl. [DAA 7ba]:	12.0	15.0	1.0
	Post-appl. [DAA 7aa]:	14.6	23.0	11.1
	Post-appl. [DAA 0aa to 10]:	11.9	16.3	12.2

ba = before application in the test item fields during the exposure period
aa = after application in the test item fields during the exposure period
DAA = days after first applications in the test item fields during the exposure period
Q_M = post-application [DAA 0aa to 28] / pre-application [DAA –2 to 0ba]

To insure that the bees were exposed to the test field and the test item detailed assessments of foraging activity were done before as well as after the application during exposure period. The daily mean foraging activity after the applications (DAA 0aa to DAA 10) was 11.9 foraging bees/minute/m2 in the test item group T1, 16.3 foraging bees/minute/m2 in the test item group T2 compared to 12.2 foraging bees/minute/m² in the control group. Furthermore, exposure of the bees was assessed by visual inspection of the *Phacelia tanacetifolia* pollen on combs entered in the hives during the study. The degree of *P. tanacetifolia* pollen from the total amount of pollen per colony ranged from 10% up to 33% during time of exposure (brood assessments on 19May06 and 25May06) at the test field in the test item group T1, from 8% up to 40% at the test field T2 and from 6% up to 85% in the control field. The results of the pollen assessments in the colonies confirm the fact that the bees were actively foraging on the test fields. A quantitative comparison between the results of the treatments is not

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possible, because the foraging and storage of pollen in a bee colony depends on outside conditions as well as on the individual need of pollen in the bee colony.

B. Observations

Honey bee mortality

The number of dead bees on the day before as well as shortly before the first application during exposure in the test fields was increased in single colonies of the test item treatment group T2 as well as in the control group. Since in both of the test fields in which this slightly increased mortality has been observed, no pre-exposure application was carried out, the temporarily increased mortality in those colonies was clearly not caused by the test item. However, on the assessments after first application during exposure (day 0 after application) the mean mortality in all treatment groups was on a low level and no test item related increase in the number of dead bees was observed (T1: 15.0 dead bees/colony, T2: 23.0 dead bees/colony, control: 29.0 dead bees). On the second application date during exposure (7 days after the first one) the mortality in all treatment groups was on a similar low level at the assessments after applications. No test item related difference in the number of dead bees of the treatment groups T1 and T2 compared to the mortality in the control group was observed at any time after the applications. This resulted in a daily mean post-application mortality (day 0 after application to day 28) in the test item treatment group T1 of 5.7 dead honey bees/colony, 6.5 dead honey bees/colony in the test group T2 and 5.6 dead honey bees/colony in the control group.

The value for QM (mean mortality post-application during exposure divided by the mean mortality pre-application during exposure) was calculated as 0.1 in the test item treatment group T1, <0.1 in the test item group T2 and 0.1 in the control group.

The mean number of dead pupae and larvae from day 0 to 28 after first application during exposure was on a low level in all treatments with 0.3 dead pupae and larvae/colony/day in the treatment group T1, 0.1 dead pupae and larvae/colony/day in the treatment group T2 and <0.1 dead pupae and larvae/colony/day in the control group. Since larval and pupae mortality was at a comparable level in all treatment groups, there were no treatment-related effects.

Honey bee flight intensity

The daily mean flight intensity (foraging bees/minute/m^2) before the first application during exposure was 15.0 in the test item treatment group T1, 13.0 in the test item treatment group T2 and 12.4 in the control group. The mean flight intensity after the applications on DAA 0 was 11.9 foraging bees/minute/m^2 in the treatment group T1, 14.4 foraging bees/minute/m^2 in the treatment group T2 as well as in the control group. On the second application date (DAA 7) no reduction in the mean flight intensity was noticed after applications in the test item group T1 and T2. The daily mean post-application (DAA 0aa to DAA 10) flight intensity was 11.9 foraging bees/minute/m^2 in the test item group T1, 16.3 foraging bees/minute/m^2 in the test item group T2 compared to 12.2 foraging bees/minute/m^2 in the control group.

The intense flight and foraging activity of the bees on the test fields was also supported by the fact that the bees stored actively *P. tanacetifolia* pollen in the combs of the colonies of all treatment groups.

Condition of the colonies and honey bee brood development

Assessments of the colony strength as judged by number of bee ways between combs filled with bees and the brood nest size (number of brood combs per colony) did not indicate significant differences between treatment groups T1, T2 and the control colonies. On the frequent assessments during exposure in the test fields and afterwards (up to 29 days after the first brood assessment) 3 colonies of the test item treatment T1 and all colonies in the test item group T2 and in the control group showed all brood stages and a similar development. One colony in the test item treatment T1 showed a lack of eggs or/and larvae on the last 2 assessment dates. In that colony a tendency to swarm or to remove the old queen was recorded (queen cells on combs) starting from the first brood assessment after set-up of the colonies at the test fields. It is very likely that this was caused by a lack of space for the colony to grow, or that the colony tried to breed a new queen because they were not satisfied with the

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old queen. Since the other 3 colonies of the treatment group T1 were in good condition over the entire test period it is very unlikely that the lack of brood was caused by the application of the test item.

<u>Honey bee behaviour in front of the colonies and within the crop area</u>
No differences regarding the behaviour of the bees were observed between the test item treatment groups T1 and T2 and the control group.

CONCLUSION

Neither test item treatments (T1 and T2) resulted in an adverse effect on honey bees as determined by mortality and flight intensity. Differences of bee behaviour between control and treatment groups were not observed. The condition of the colonies as assessed by colony strength and size of the brood nest was not affected by any of the treatments. From the detailed assessment of the honey bee brood status one colony in the test item treatment group T1 showed a lack of brood on the last assessments. In that colony a tendency to swarm or to remove the old queen was recorded (queen cells on combs) starting from the first brood assessment after set-up of the colonies at the test fields. It is very likely that this was caused by a lack of space for the colony to grow, or that the colony tried to breed a new queen because they were not satisfied with the old queen. No evidence of an irritation or termination of the development based upon exposure to treated crops was obtained in the other colonies of the test group T1 as well as in the test item group T2 and the control colonies.

([redacted] A., 2006)

Report:	**KIIIA1 10.4.5/02, [redacted] J., [redacted] Ch., [redacted] H.J. & [redacted] T.; 2008a**
Title:	Assessment of effects of Spirotetramat OD 150 to honey bee (Apis mellifera) colonies under a realistic field scenario in a melon crop - GLP Trial 2008 -. Date: 2008-07-04
Organisation:	Bayer CropScience AG, [redacted], Germany
Report No.:	MAUS/AM045; M-303607-01-1
Publication:	unpublished
Dates of experimental work:	January 25, 2008 - March 19, 2008
Guidelines:	EPPO 170 (3)
Deviations:	no major deviations
GLP	yes (certified laboratory)

Executive summary

This study aimed to determine potential effects of two different application scenarios of Spirotetramat OD 150 under realistic field conditions in a melon crop to honey bee (*Apis mellifera*) colonies. A special focus was made on potential brood effects.

The test item Spirotetramat OD 150 was applied during bee flight in flowering melon (*Cucumis melo*) fields of approximately 1 ha size in 250 L water/ha. There were 3 treatment groups, each with 3 replicates. Treatment group 1 (plots 6, 8 and 9) served as untreated control. Treatment group 2 (plots 1, 2 and 4) received 4 applications with 75 g a.s./ha in spray intervals of 7 days. Treatment group 3 (plots 3, 5 and 10) received 2 applications with 88 g a.s./ha in spray intervals of 7 days. All plots were in approximately 3 km distance of each other.

Two bee hives were set up on each plot. Each hive contained a colony of approximately 18,000 bees (*Apis mellifera mellifera* L.) plus a queen of the same maternal origin (sister queens) at the start of the study and comprised of 7 frames for brood of all ages and 1 honey frame. The hives were set up on the plots 2.5 weeks before the first brood assessment (T0, pre-treatment assessment) was conducted and remained there until the last weekly in-field brood assessment (i.e., T2 for treatment group 3 and T4 for treatment group 2) was conducted. Thereafter the colonies were transferred to an area of less intensive agricultural use (without additional pesticide exposure). The last brood assessment was conducted 2.5

weeks after the last application had been conducted (i.e., T3 for treatment group 3 and T5 for treatment group 2). Control colonies were assessed in parallel to the assessment dates of the respective treated groups. During the Tx assessments the bee colonies were observed for potential treatment-related effects on brood (eggs, unsealed brood – larvae, and sealed brood - pupae), as well as hive weight, colony strength, pollen and nectar/honey storage.
Additionally, the number of foraging bees, returning to the hive with and without pollen loads, as well as mortality in front of each hive were assessed in 48-hour intervals throughout the study. Furthermore (according to the same time table) the number of melon blossoms was counted exemplarily for 10 x 10 m rows on each plot throughout the study. Concurrently, the number of blossoms visited by bees was assessed.
The comb area containing brood of all stages (eggs, larvae and pupae) fluctuated in the control as well as in treatment group 2 (4 x 75 g a.s./ha) and in treatment group 3 (2 x 88 g a.s./ha) on the different assessment days, reflecting the typical natural variability of this endpoint. No treatment-related effect on brood and abundance of adult bees was found.
Foraging activity (bees returning to the hives with and without pollen loads) and hive weight development were unaffected in the control and both Spirotetramat treatment groups.
No effect on the storage behaviour of the honeybees regarding pollen and nectar/honey was found. According to visual observations made by the principal investigator during the hive assessments Tx, approximately 60% of the pollen stored in the combs originated form melon blossoms.
No treatment-related effect was found on mortality of adult honeybees or on the number of dead larvae and pupae found in front of the bee hives.
No effect on brood development (eggs, larvae, pupae) and abundance of adult honeybees was found after the application of 4 x 75 g Spirotetramat/ha or 2 x 88 g Spirotetramat/ha (spray intervals of 7 days) in flowering melon. Likewise, no effects on foraging activity, mortality determined in front of the hives, hive weight development or the storage behaviour of honeybee colonies were found.

MATERIAL AND METHODS

A Materials

1. Test material	Spirotetramat OD 150
Description	beige suspension
Lot/batch No.	batch No.: 2007-006494, specification No.: 102000016434
Content a.s.	152.8 g/L
Stability of test compound	Approved until 2009-09-17 when stored at room temperature (25 ± 5°C)
2. Vehicle and/or positive control	Water
3. Test animals	
Species	The test species was *Apis mellifera mellifera* L. The colonies (18 in total) had approximately 18,000 adult bees per colony at test initiation. They were homogenous regarding population, colony strength, food storage, brood status and preparation. Preparation of the colonies (not under GLP) started in an appropriate temporal distance to the beginning of the study. The hives consisted of 8 frames, comprising of 7 frames for broods of all ages and 1 honey frame. The queens were of the same maternal origin (sister queens) and were of the same age (born in November 2007).

B Study design and methods

1. In life dates	January 25, 2008 - March 19, 2008
2. Experimental treatments	

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The study was carried out in the surroundings of [redacted] (province of San Juan, Argentina). Nine plots of approx. 1 ha each were used in this study. All experimental plots were approximately 3 km distant from each other. The crop used was melon *Cucumis melo* [Cucurbitaceae], variety "Honeydew green flesh" (SEMINI). No flowering bee-attractive crops or greater populations of blooming bee-attractive weeds were reported at distances near the crop where bees might leave the treated or control area to forage.

The test item Spirotetramat OD 150 was applied during bee flight in the flowering melon crop fields in 250 L water/ha. There were 3 treatment groups, each with 3 replicates. Treatment group 1 (plots 6, 8 and 9) served as untreated control. Treatment group 2 (plots 1, 2 and 4) received 4 applications with 75 g a.s./ha in spray intervals of 7 days. Treatment group 3 (plots 3, 5 and 10) received 2 applications with 88 g a.s./ha in spray intervals of 7 days.

The bee colonies were placed in a distance of at least 1.5 m from each other in the middle of each plot 10 days before the start of flowering of the melon crop (acclimatisation = pre-exposure period). The colonies remained on the plots for the pre-exposure and exposure period, i.e., 4 weeks after the first application for treatment group 2 (= up to 1 week after the last application) as well as control, and 2 weeks after the first application for treatment group 3 (= up to 1 week after the last application). Thereafter, the colonies were taken to an area of less intensive agricultural use (with no additional pesticide exposure) where they were set up.

3. Observations

<u>Brood Development:</u>

The percentages of the comb area in each hive occupied with cells containing eggs, larvae (worker brood unsealed cells) or pupae (worker brood sealed cells) were separately assessed during the Tx assessments by visual estimation of the beekeeper. The first brood assessment (T0) was done pre-exposure, on 2008-02-03/04, one/two days before the first application of each treatment scenario was performed. After the first application per treatment scenario was conducted, the brood assessments were done in weekly intervals (7 days ± 1 day) but always at latest on the day before the next application was conducted. For as long as the colonies were set up in the melon fields, 4 further brood assessments (T1 to T4) were conducted in treatment group 2, 2 further brood assessments (T1 to T2) were conducted for treatment group 3 and 5, and 2 further brood assessments (T1 to T5) were conducted for the control. The final brood assessments in the control (Final = T6), treatment group 2 (Final = T5) and treatment group 3 (Final = T3) were performed 2-3 weeks after the last application was conducted when the bees were not longer set up in the melon fields.

<u>Colony Strength</u>

The number of adult bees living in each hive was visually estimated by the beekeeper during the Tx assessments. Likewise, the percentage of each comb side covered by adult bees was assessed while extracting the comb of the hive. Colony strength was assessed on the same dates as for the brood evaluation.

<u>Weight of the Bee Hives</u>

The bee hive weight was determined gravimetrically during the assessments carried out on the respective Tx assessment dates for each treatment group.

<u>Pollen and Nectar/Honey Stores</u>

The percentage of comb area occupied by stored pollen and nectar/honey in each hive was visually estimated during the Tx assessments.

<u>Foraging Activity of the Bees in the Crop</u>

Depending on the weather conditions, foraging activity on the plots was determined. The number of melon blossoms, on which honeybees were found foraging and the number of melon blossoms without foraging bees was determined along 10 rows 10 m long that were impartially selected within each plot. Assessments were carried out in 48 h intervals (except for dates, when Tx assessments were performed) in each treatment group from approximately one week before the first application was performed until 4 days after the

last application in each treatment group. The following number of assessments was performed: 4 before the 1st application, 3 between after the 1^{st} and before the 2^{nd} application, 2 between after the 2^{nd} and before the 3^{rd} application, 2 after the 3^{rd} and before the 4^{th} application (except for treatment group 3), and 2 after the 4^{th} application (except for treatment group 3).

Mortality

The mortality of adult bees (worker and drones), larvae and pupae was recorded by collecting and counting their numbers found in the dead bee traps attached to the hive. Dead bees, drones, larvae and pupae were immediately removed from the dead bee trap after having been recorded. Special attention was paid to larvae, pupae and malformed bees that were expelled from the hive. Assessments were carried out in 48 h intervals (except for dates, when Tx assessments were performed) in each treatment group from approximately one week before the first application was performed until 4 days after the last application in each treatment group. The following number of assessments was performed: 4 before the 1^{st} application, 3 between after the 1^{st} and before the 2^{nd} application, 2 between after the 2^{nd} and before the 3^{rd} application, 2 after the 3^{rd} and before the 4^{th} application (except for treatment group 3), and 2 after the 4th application (except for treatment group 3).

Returning of Foraging Bees to the Beehives

Bees entering the hives with and without pollen loads were counted when they arrived at the landing board for a 1-minute interval. Assessments were carried out in 48 h intervals (except for dates, when Tx assessments were performed) in each treatment group from approximately one week before the first application was performed until 4 days after the last application in each treatment group. The following number of assessments was performed: 4 before the 1^{st} application, 3 between after the 1^{st} and before the 2^{nd} application, 2 between after the 2^{nd} and before the 3^{rd} application, 2 after the 3^{rd} and before the 4^{th} application (except for treatment group 3), and 2 after the 4^{th} application (except for treatment group 3).

Behaviour

Possible behavioural anomalies of the bees were observed and recorded at the same times as the observations of foraging activity were conducted.

Blossom Counting

During the assessments for the determination of Foraging Activity of the Bees in the Crop also the blossom density was assessed. This was done by counting the number of melon blossoms, on which honeybees were found foraging and the number of melon blossoms without foraging bees, determined along 10 rows 10 m long that were impartially selected within each plot (different areas on the plots for each assessment). Assessments were carried out in 48 h intervals (except for dates, when Tx assessments were performed) in each treatment group from approximately one week before the first application was performed until 4 days after the last application in each treatment group. The following number of assessments was performed: 4 before the 1^{st} application, 3 between after the 1^{st} and before the 2^{nd} application, 2 between after the 2^{nd} and before the 3^{rd} application, 2 after the 3^{rd} and before the 4^{th} application (except for treatment group 3), and 2 after the 4^{th} application (except for treatment group 3).

Climatic conditions (non-GLP data) were recorded at the time of applications and at the bee activity assessments throughout the study. Temperature and relative air humidity were measured. Cloudiness (eights of the sky covered with clouds) was estimated by the observer. No statistical evaluation was performed.

RESULTS AND DISCUSSION

A. Findings

Assess-ment	Parameter	Treatment group 1 (Control)			Treatment group 2 (4 x 75 g a.s./ha)			Treatment group 3 (2 x 88 g a.s./ha)		
		Plot No. 8	Plot No. 6	Plot No. 9	Plot No. 4	Plot No. 2	Plot No. 1	Plot No. 5	Plot No. 10	Plot No. 3
		% comb area per assessment as average of all hives per plot								
T0	Egg deposition	7.2	3.9	6.1	4.1	7.7	6.1	7.3	7.2	3.8
T1		4.7	7.5	9.1	7.8	5.8	8.1	9.1	11.4	6.6
T2		5.8	1.6	6.6	3.9	6.9	3.9	5.8	5.6	5.0
T3		6.4	5.8	6.7	5.3	5.8	5.6	-	-	-
T4		9.8	4.7	6.3	4.7	5.5	4.8	-	-	-
T5		7.2	4.1	6.9	-	-	-	7.0	4.5	8.8
T6		5.8	5.3	5.6	3.4	3.6	5.9	-	-	-
T0	Unsealed brood	10.8	8.9	12.3	6.9	6.3	8.6	8.9	9.8	12.7
T1		10.8	9.2	14.1	8.0	8.0	11.1	16.1	9.2	11.1
T2		8.8	6.6	12.2	6.6	8.9	10.0	10.3	12.8	10.2
T3		10.9	6.4	11.3	9.2	8.8	7.7	-	-	-
T4		13.3	8.9	12.0	9.5	9.5	7.2	-	-	-
T5		12.0	6.6	9.5	-	-	-	8.4	5.8	9.5
T6		10.0	8.1	7.7	7.8	6.1	4.5	-	-	-
T0	Sealed brood	15.3	22.5	24.2	14.2	5.5	12.5	10.3	9.7	32.7
T1		27.2	25.5	26.3	14.1	11.7	16.1	16.4	16.1	33.9
T2		24.1	20.9	26.9	16.7	14.2	22.2	25.3	20.5	25.6
T3		23.0	16.9	25.6	19.5	18.8	22.3	-	-	-
T4		23.8	14.7	23.9	17.7	20.0	20.5	-	-	-
T5		28.8	17.8	27.3	-	-	-	23.0	22.0	24.8
T6		21.7	15.6	19.4	14.5	16.1	8.0	-	-	-
T0	Comb area covered by adult bees	75.6	71.9	72.2	80.9	85.8	81.9	73.4	79.4	74.7
T1		78.8	75.9	70.9	86.9	84.4	78.3	75.6	82.5	82.8
T2		75.9	75.6	71.3	81.6	80.3	79.1	73.1	76.3	81.9
T3		80.6	81.3	79.7	83.4	70.9	84.7	-	-	-
T4		75.9	75.0	74.1	77.5	68.9	80.3	-	-	-
T5		78.4	78.8	76.6	-	-	-	70.9	71.6	81.3
T6		70.6	70.0	66.6	77.8	62.5	73.1	-	-	-
T0	Pollen stores	8.9	6.3	9.1	6.3	12.3	9.8	4.5	2.2	11.1
T1		11.6	4.1	6.4	6.3	12.8	8.8	6.6	8.4	9.8
T2		12.2	6.7	10.9	8.6	14.1	11.6	9.5	6.4	8.0
T3		8.9	6.1	9.1	6.4	10.9	8.6	-	-	-
T4		9.2	5.9	11.3	10.8	10.8	12.5	-	-	-
T5		2.7	4.5	6.7	-	-	-	5.3	7.0	8.6
T6		12.2	5.9	10.8	10.0	10.9	13.6	-	-	-
T0	Nectar / honey stores	28.9	48.7	35.5	47.5	36.7	43.8	38.6	38.0	27.3
T1		33.1	46.4	36.6	50.9	35.5	43.9	40.5	38.1	30.9
T2		34.5	56.1	40.2	51.4	34.5	44.7	40.0	37.0	37.7
T3		28.1	52.5	33.8	43.6	39.1	44.2	-	-	-
T4		30.5	51.6	37.5	46.9	42.3	48.1	-	-	-
T5		29.8	53.1	34.5	-	-	-	33.1	33.8	34.1
T6		30.6	47.8	31.9	42.7	36.7	42.3	-	-	-
		Estimated No. of adults [n] as average of all hives per plot								
T0	Estimated No. of adult honeybees	19,000	18,500	18,500	19,000	19,000	19,000	18,750	19,000	19,000
T1		19,000	19,000	18,750	19,000	19,000	19,000	18,500	19,000	19,000
T2		19,250	19,250	19,250	19,500	19,250	19,250	19,000	19,000	19,500
T3		19,500	20,000	19,500	19,750	19,500	20,000	-	-	-

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Assess-ment	Parameter	Treatment group 1 (Control)			Treatment group 2 (4 x 75 g a.s./ha)			Treatment group 3 (2 x 88 g a.s./ha)		
		Plot No. 8	Plot No. 6	Plot No. 9	Plot No. 4	Plot No. 2	Plot No. 1	Plot No. 5	Plot No. 10	Plot No. 3
T4		19,500	20,000	20,000	19,750	19,750	19,750	-	-	-
T5		19,750	20,500	20,250	-	-		20,000	20,000	20,500
T6		20,500	21,250	21,000	21,000	20,750	21,000	-	-	-
		No. as average of all hives per plot [n]								
Σ before 1st appl.	Dead worker bees in front of hive	5.0	4.5	4.5	2.0	2.5	2.5	7.0	5.0	1.0
Σ after 1st - before 2nd appl.		2.5	4.0	0.0	1.5	1.0	0.0	2.0	3.5	0.5
Σ after 2nd - before 3rd appl.		1.0	0.0	0.5	0.0	2.0	1.5	2.5	0.0	0.0
Σ after 3rd - before 4th appl.		0.0	7.0	2.0	0.5	0.5	0.0	-	-	-
Σ after 4th appl.		0.0	0.5	0.0	3.5	0.0	1.0	-	-	-
Σ after all appl.		3.5	11.5	2.5	5.5	3.5	2.5	4.5	3.5	0.5
Σ before 1st appl.	Dead pupae in front of hive	0.0	0.0	0.5	0.0	0.0	0.5	0.0	1.5	0.0
Σ after all appl.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
Σ before 1st appl.	Dead larvae in front of hive	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Σ after all appl.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		No. as average of all hives per plot [n/min]								
Ø before 1st appl.	Returning bees with pollen	8.5	7.8	13.2	6.0	9.5	12.5	9.4	11.6	23.6
Ø 1st - 2nd appl.		18.0	6.7	5.8	11.5	6.3	13.5	7.8	19.0	16.7
Ø 2nd - 3rd appl.		21.5	16.0	13.5	26.3	8.0	13.5	15.5	22.5	22.5
Ø 3rd - 4th appl.		30.0	2.3	11.3	15.3	14.3	19.3	-	-	-
Ø after 4th appl.		19.3	11.3	14.3	12.3	15.8	12.8	-	-	-
		No. as average of all hives per plot [n/min]								
Ø before 1st appl.	Returning bees without pollen	13.0	9.5	14.5	8.8	7.3	6.8	9.5	13.8	13.4
Ø 1st - 2nd appl.		19.7	9.8	9.3	12.2	13.0	16.8	8.0	16.7	20.5
Ø 2nd - 3rd appl.		23.8	16.8	12.3	18.8	10.0	15.0	17.0	18.3	21.8
Ø 3rd - 4th appl.		16.8	9.8	15.0	14.3	18.0	24.3	-	-	-
Ø after 4th appl.		27.0	21.3	24.0	17.5	26.5	21.8	-	-	-
		Hive weight development [kg] as average of all hives per plot								
T0	Hive weight	21.6	27.0	25.5	25.5	22.5	24.8	23.8	22.7	23.4
T1		22.2	27.7	26.4	26.5	22.5	25.9	24.1	23.0	24.5

Assess-ment	Parameter	Treatment group 1 (Control)			Treatment group 2 (4 x 75 g a.s./ha)			Treatment group 3 (2 x 88 g a.s./ha)		
		Plot No. 8	Plot No. 6	Plot No. 9	Plot No. 4	Plot No. 2	Plot No. 1	Plot No. 5	Plot No. 10	Plot No. 3
T2		22.5	28.6	27.1	26.6	22.6	27.1	25.2	24.2	25.8
T3		22.0	28.3	25.5	25.7	24.0	25.9	-	-	-
T4		21.7	28.4	25.7	25.9	24.8	27.3	-	-	-
T5		21.3	27.6	24.6	-	-	-	23.1	24.0	23.7
T6		21.2	25.9	24.2	24.6	23.2	25.0	-	-	-

T1 – T6: assessment days -: not assessed
Application dates: 1 day after respective Tx assessment beginning from T0 up to T3 for treatment group 2 and beginning from T0 up to T1 for treatment group 3
T0: 1 day prior to 1st application T1: 7 to 8 days after 1st application
T2: 14 to 15 days after 1st application T3: 20 days after 1st application
T4: 27 days after 1st application T5: 33 days after 1st application
T6: 42 days after 1st application

B. Observations

Honey bee brood

The comb area containing brood of all stages (eggs, larvae and pupae) fluctuated in the control as well as in treatment group 2 (4 x 75 g a.s./ha) and in treatment group 3 (2 x 88 g a.s./ha) on the different assessment days, reflecting the typical natural variability of this endpoint. No treatment-related effect on brood and abundance of adult bees was found.

Foraging activity

Foraging activity (bees returning to the hives with and without pollen loads) and hive weight development were unaffected in the control and both Spirotetramat treatment groups

Storage behaviour

No effect on the storage behaviour of the honeybees regarding pollen and nectar/honey was found. According to visual observations made by the principal investigator during the hive assessments Tx, approximately 60% of the pollen stored in the combs originated form melon blossoms.

Mortality

No treatment-related effect was found on mortality of adult honeybees as well as on the number of dead larvae and pupae found in front of the bee hives.

CONCLUSION

No effect on brood development (eggs, larvae, pupae) and abundance of adult honeybees was found after the application of 4 x 75 g Spirotetramat/ha or 2 x 88 g Spirotetramat/ha (spray intervals of 7 days) in flowering melon. Likewise, no effects on foraging activity, mortality determined in front of the hives, hive weight development and the storage behaviour of honeybee colonies were found.

([redacted] J. et al., 2008a)

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Report:	**KIIIA1 10.4.5/03, [redacted] T., [redacted] Ch., [redacted] H.J. & [redacted] J.; 2008**
Title:	Assessment of effects of Spirotetramat OD 150 on honey bee (*Apis mellifera* L.) colonies under a realistic field scenario in a melon crop in 2007 (non-GLP). Date: 2008-05-19
Organisation:	Bayer CropScience AG, [redacted], Germany
Report No.:	MAUS/AM044; M-301729-01-1
Publication:	unpublished
Dates of experimental work:	February 05, 2007 - March 21, 2007
Guidelines:	Special design, partly following EPPO 170 (3)
Deviations:	not applicable
GLP	no

Executive summary

This study aimed to determine potential effects of two different application scenarios of Spirotetramat OD 150 under realistic field conditions in a melon crop to honey bee (Apis mellifera) colonies. A special focus was made on potential brood effects.

The test item Spirotetramat OD 150 was applied during bee flight in flowering melon (*Cucumis melo*) fields of approximately 1 ha in 200 L water/ha. There were 3 treatment groups, each with 3 replicates. Treatment group 1 (plots 2, 3 and 7) served as tap water treated control with applications in intervals of 7, 8 and 12 days. Treatment group 2 (plots 1, 5 and 9) received 4 applications with 72 g a.s./ha in intervals of 7, 8 and 12 days. Treatment group 3 (plots 4, 6 and 8) received 4 applications with 88 g a.s./ha in intervals of 7, 8 and 12 days. All plots were in approximately 3 km distance of each other.

Four bee hives were set up on each plot. Each hive contained approximately 15,000 bees (*Apis mellifera mellifera* L.) plus a queen of the same maternal origin (sister queens) and comprised of 5 frames for brood of all ages, 1 honey frame, 3 empty frames and 1 empty feeding frame. The hives were set up on the plots 2 weeks before the first brood assessment (T0) was conducted and remained there until the last brood assessment (T4) was conducted, except for 50% of the hives, which had to be removed after the T3 assessment due to decreasing density of blossoms of the crop after this point of time.

The bee colonies were observed for potential treatment-related effects on brood (eggs, unsealed brood – larvae, and sealed brood - pupae), as well as hive weight, colony strength, pollen and nectar/honey storage. Additionally the number of foraging bees returning to the hive with and without pollen loads as well as mortality in front of each hive were assessed. Furthermore the number of melon blossoms was counted exemplarily for a 10 times 10 m rows on each plot throughout the study. During this assessment additionally the number of blossoms visited by bees was determined.

The comb area containing brood of all stages (eggs, larvae and pupae) fluctuated in the control as well as in treatment group 2 (4 x 72 g a.s./ha) and in treatment group 3 (4 x 88 g a.s./ha) on the different assessment days, indicating the typical natural variability of this endpoint. No treatment-related effect on brood and abundance of adult bees was found.

Foraging activity (bees returning to the hives with and without pollen loads) and hive weight development were unaffected in the control and both Spirotetramat treatment groups.

No effect on the storage behaviour of the honeybees regarding pollen and nectar/honey was found.

No treatment-related effect was found on mortality of adult honeybees as well as on the number of dead larvae and pupae found in front of the bee hives.

No effect on brood development (eggs, larvae, pupae) and abundance of adult honeybees was found after the application of 4 x 72 g Spirotetramat/ha and 4 x 88 g Spirotetramat/ha (intervals of 7, 8 and 12 days) in flowering melon. Likewise, no effects on foraging activity, mortality determined in front of the hives, hive weight development and the storage behaviour of honeybee colonies were found. However, due to a certain uncertainty about exposure in the period after the 2nd application, a positive proof of the absence of effects in spite of full exposure to the treated crop is only given for the study period until the T1 assessment.

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MATERIAL AND METHODS

A Materials

1. Test material	Spirotetramat OD 150
Description	light brown suspension
Lot/batch No.	batch No.: 2007-000083, Material No.: 06424376 Specification No.:102000011380
Content a.s.	149.5 g/L
Stability of test compound	Approved until 2009-01-24 when stored at room temperature (25 ± 5°C)
2. Vehicle and/or positive control	Water
3. Test animals	
Species	The test species was *Apis mellifera mellifera* L. The colonies (36 in total) had approximately 15,000 adult bees per colony at test initiation. They were homogenous regarding population, colony strength, food storage, brood status and preparation. Preparation of the colonies started in an appropriate temporal distance to the beginning of the study. The hives consisted of 10 frames, comprising of 5 frames for broods of all ages, 1 honey frame, 3 empty frames and 1 empty feeding frame. The queens were of the same maternal origin (sister queens) and were of the same age.

B Study design and methods

1. In life dates	February 05, 2007 - March 21, 2007

2. Experimental treatments

The study was carried out in the surroundings of [redacted] (province of San Juan, Argentina). Nine plots of approx. 1 ha each were used in this study. All experimental plots were approximately 3 km distant from each other. The crop used was melon *Cucumis melo* [Cucurbitaceae], variety "Early spring" (SEMINI).

The test item Spirotetramat OD 150 was applied during bee flight in flowering melon (*Cucumis melo*) fields of approximately 1 ha in 200 L water/ha. There were 3 treatment groups, each with 3 replicates. Treatment group 1 (plots 2, 3 and 7) served as tap water treated control with applications in intervals of 7, 8 and 12 days. Treatment group 2 (plots 1, 5 and 9) received 4 applications with 72 g a.s./ha in intervals of 7, 8 and 12 days. Treatment group 3 (plots 4, 6 and 8) received 4 applications with 88 g a.s./ha in intervals of 7, 8 and 12 days.

The bee colonies were impartially assigned to the treatment groups, 4 per plot, using a computer generated random list. One additional hive was placed outside of the trial range and kept as a reserve hive until the end of the acclimatization period. In the time after the 2nd application, blossom density of the crop at least temporarily decreased on most of the plots due to crop maintenance (irrigation) issues, and went below a level where the plots could still provide sufficient forage for all colonies set up on the plots, and where sufficient crop attractiveness for bees was still assured. Therefore, on 2007-03-12 two bee hives were removed from each plot and placed outside the study range. Hives to be removed were selected according their relative population strength and to their condition (weaker colonies were removed). The remaining hives were removed from the plots on 2007-03-21, at study end.

3. Observations

<u>Brood Development</u>:

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The percentages of the comb area in each hive occupied with cells containing eggs, larvae (worker brood unsealed cells) or pupae (worker brood sealed cells) were separately assessed during the T_0 to T_4 assessments by the beekeeper.

Colony Strength

The number of adult bees living in each hive was visually estimated by the beekeeper during the T_0 to T_4 assessments. Likewise, the percentage of each comb side covered by adult bees was assessed while extracting the comb of the hive. Colony strength was assessed on the same dates as for the brood evaluation.

Weight of the Bee Hives

The device for weighing the bee hives was an electronic balance.

Pollen and Nectar/Honey Stores

The percentage of comb area occupied by stored pollen and nectar/honey in each hive was visually estimated during the T_0 to T_4 assessments.

Foraging Activity of the Bees in the Crop

Depending on the weather conditions, foraging activity on the plots was determined. The number of melon blossoms, on which honeybees were found foraging and the number of melon blossoms without foraging bees was determined along 10 times 10 m rows impartially selected within each plot.

Mortality

The mortality of bees was recorded by collecting and counting dead bees found on the fine white nets placed on the ground in front of each hive. This procedure was carried out approximately every 48 h in concordance with the assessment of "Returning of foraging bees to the beehives". Dead bees were immediately removed from the net after having been recorded. Special attention was paid to larvae, pupae and malformed bees that were expelled from the hive.

Returning of Foraging Bees to the Beehives

Bees entering the hives with and without pollen loads were counted when they arrived at the landing board for a 1-minute interval. Assessments were carried out in approximately 48 h intervals.

Behaviour

Possible behavioural anomalies of the bees were observed and recorded at the same times as the observations of foraging activity were conducted.

Blossom Counting

During the assessments for the determination of foraging activity of bees in the crop also the blossom density was assessed. This was done by counting the number of melon blossoms, on which honeybees were found foraging and the number of melon blossoms without foraging bees, determined along 10 times 10 m rows impartially selected within each plot.

Climatic Conditions

Climatic conditions were recorded at the time of the bee activity assessments throughout the study. Temperature and relative air humidity were measured. Cloudiness (eights of the sky covered with clouds) was estimated by the observer.

No statistical evaluation was performed.

RESULTS AND DISCUSSION

A. Findings

Assess-ment	Parameter	Treatment group 1 (Control)			Treatment group 2 (4 x 72 g a.s./ha)			Treatment group 3 (4 x 88 g a.s./ha)		
		Plot No. 2	Plot No. 3	Plot No. 7	Plot No. 1	Plot No. 5	Plot No. 9	Plot No. 4	Plot No. 6	Plot No. 8
		Average per hive in % comb area per assessment								
T0	Egg deposition	9.3	8.9	11.3	9.0	7.9	6.5	6.4	6.3	11.8
T1		7.7	5.8	4.3	3.1	6.0	4.2	3.6	5.7	6.0
T2		8.3	5.8	7.4	5.6	5.7	5.3	9.5	8.5	5.9
T3		5.6	5.1	6.3	4.4	5.3	4.4	8.8	6.9	4.7
T4		5.3	3.5	4.9	4.4	3.9	3.9	6.8	4.3	4.2
T0	Unsealed brood	13.2	10.6	7.3	10.1	4.2	6.1	5.8	4.0	4.2
T1		10.8	7.4	7.8	10.8	5.1	7.6	6.0	6.3	9.9
T2		10.1	7.4	8.7	6.6	7.2	9.7	5.0	6.9	8.2
T3		12.2	8.7	8.1	7.2	7.8	10.9	10.1	8.5	7.8
T4		8.1	9.9	7.1	6.8	8.1	8.1	8.3	7.9	7.1
T0	Sealed brood	4.1	3.4	6.2	4.4	5.7	9.3	3.4	5.1	5.6
T1		17.3	10.2	14.0	14.0	8.4	12.9	7.8	9.3	11.3
T2		21.8	11.9	16.2	20.5	11.9	15.8	10.2	12.6	18.2
T3		16.6	13.1	14.6	16.3	12.2	14.7	12.0	14.8	18.5
T4		20.8	19.3	17.8	18.6	17.6	15.7	14.9	20.1	15.3
T0	Comb area covered by adult bees	58.9	54.7	61.6	55.7	59.2	64.6	59.4	65.6	66.2
T1		61.0	64.3	64.2	51.4	60.8	60.0	58.6	64.3	71.1
T2		67.4	68.2	71.1	67.2	66.3	72.8	65.3	70.1	77.3
T3		76.3	72.1	73.9	74.0	65.5	75.1	68.9	70.2	77.5
T4		71.7	67.8	72.8	79.7	77.2	67.2	74.2	75.3	78.1
T0	Pollen stores	3.7	7.0	5.8	3.8	4.4	4.4	5.7	3.1	8.4
T1		1.6	2.9	3.9	1.4	3.6	3.8	4.9	3.8	5.8
T2		4.0	4.9	7.6	3.4	4.4	6.4	7.2	4.7	7.5
T3		12.7	7.7	8.8	5.6	14.5	7.2	6.5	9.7	9.0
T4		5.4	8.5	10.8	8.1	10.7	8.9	9.4	8.1	11.3
T0	Nectar / honey stores	36.4	30.4	31.5	41.9	29.9	38.3	40.6	32.8	33.7
T1		28.7	28.5	32.9	35.7	34.4	41.5	33.8	39.6	37.0
T2		29.2	31.8	36.0	35.4	32.0	39.3	34.5	35.6	39.3
T3		28.0	30.9	34.9	39.1	31.3	38.8	30.5	32.4	38.7
T4		40.3	33.2	46.3	46.0	43.5	41.1	34.4	31.7	49.0
		Average No. per assessment per hive [n]								
T0	Estimated No. of adult honeybees	16,500	15,750	16,500	17,000	15,500	14,500	16,500	16,000	16,000
T1		17,500	17,000	17,250	16,500	16,250	16,750	16,250	17,000	17,250
T2		17,250	16,750	17,500	17,250	16,250	17,000	17,000	17,000	18,000
T3		18,000	17,750	17,750	17,750	17,000	17,000	17,000	17,000	18,000
T4		16,500	17,000	18,000	17,500	18,000	16,500	17,500	18,000	18,000
		Average No. per assessment per hive [n]								
Σ 1st - 3rd appl.	Dead worker bees in front of hive	48.8	14.0	10.0	152.8 [11]	14.5	19.3	29.3	32.0	25.3
Σ after 4th appl.		59.5	9.0	3.5	8.0	1.5	45.5	6.5	6.5	6.0
Σ 1st - 3rd appl.	Dead pupae in front of hive	0.0	0.0	0.0	0.3	0.0	0.0	0.3	0.0	0.0
Σ after 4th appl.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[11] Increased adult mortality was caused by punctual inter-colony aggression and is therefore not considered treatment-related.

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Assess-ment	Parameter	Treatment group 1 (Control)			Treatment group 2 (4 x 72 g a.s./ha)			Treatment group 3 (4 x 88 g a.s./ha)		
		Plot No. 2	Plot No. 3	Plot No. 7	Plot No. 1	Plot No. 5	Plot No. 9	Plot No. 4	Plot No. 6	Plot No. 8
Σ 1st - 3rd appl.	Dead larvae in front of hive	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0
Σ after 4th appl.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Average number per assessment per hive [n]								
Ø 1st - 2nd appl.	Returning bees with pollen	16.6	16.9	26.4	17.4	16.1	16.8	7.3	21.6	13.8
Ø 2nd - 3rd appl.		29.0	15.1	29.0	18.5	15.4	18.4	10.5	22.4	29.5
Ø 3rd - 4th appl.		19.9	18.9	21.3	16.8	18.2	18.3	16.8	19.4	19.7
Ø after 4th appl.		13.3	16.3	10.5	10.3	14.0	12.3	13.8	19.3	11.5
Ø 1st - 2nd appl.	Returning bees without pollen	19.4	15.8	12.8	13.8	10.5	15.8	6.0	10.4	22.8
Ø 2nd - 3rd appl.		16.4	16.4	18.6	16.4	16.6	14.1	12.4	14.9	23.9
Ø 3rd - 4th appl.		23.0	21.1	23.5	20.0	18.6	15.2	14.9	17.4	26.5
Ø after 4th appl.		35.8	33.0	30.3	27.0	30.0	15.5	23.3	21.3	28.0
		Average hive weight per assessment [kg]								
T0	Hive weight	26.1	25.0	25.5	26.9	24.2	25.4	25.2	25.5	24.7
T1		26.9	25.3	26.3	27.2	25.8	27.2	24.7	25.9	26.5
T2		25.3	25.3	26.6	27.5	24.7	27.2	24.4	25.6	27.1
T3		25.5	25.3	26.7	27.7	25.1	26.9	23.9	25.4	27.7
T4		28.5	26.1	28.5	30.0	27.9	27.6	24.7	25.2	30.0

T1 – T4: assessment days
T0: 3 to 4 days prior to 1st application
T1: 7 to 8 days after 1st application
T2: 15 to 16 days after 1st application
T3: 23 to 24 days after 1st application
T4: 35 to 36 days after 1st application

B. Observations

Honey bee brood

The comb area containing brood of all stages (eggs, larvae and pupae) fluctuated in the control as well as in treatment group 2 (4 x 72 g a.s./ha) and in treatment group 3 (4 x 88 g a.s./ha) on the different assessment days, indicating the typical natural variability of this endpoint. No treatment-related effect on brood and abundance of adult bees was found.

Foraging activity

Foraging activity (bees returning to the hives with and without pollen loads) and hive weight development were unaffected in the control and both Spirotetramat treatment groups.

Storage behaviour

No effect on the storage behaviour of the honeybees regarding pollen and nectar/honey was found.

Mortality

No treatment-related effect was found on mortality of adult honeybees as well as on the number of dead larvae and pupae found in front of the bee hives.

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Remark

A flower density equivalent to a number of approximately 200 melon blossoms per 100 m row in a melon field of the respective size is judged to be sufficient to provide an attractive food source for the honeybees. At a lower blossom density the food supply may not be sufficient and the bees might forage on other sources outside the study plots. In the trial reported here, the blossom density fell at least temporarily below this critical value on most of the study plots after the 2nd application due to problems with crop maintenance. Therefore, crop attractiveness to bees was at least for a part of the study period diminished on the affected plots, so that a maximum exposure of the bees was not assured for this time. However, at least until brood assessment T1 there was full exposure of the bees to the treated crop.

Conclusion

No effect on brood development (eggs, larvae, pupae) and abundance of adult honeybees was found after the application of 4 x 72 g spirotetramat/ha and 4 x 88 g spirotetramat/ha (intervals of 7, 8 and 12 days) in flowering melon. Likewise, no effects on foraging activity, mortality determined in front of the hives, hive weight development and the storage behaviour of honeybee colonies were found. However, due to a certain uncertainty about exposure in the period after the 2nd application, a positive proof of the absence of effects in spite of full exposure to the treated crop is only given for the study period until the T1 assessment.

([redacted], 2008)

Report:	**KIIIA1 10.4.5/04, [redacted]; 2008**
Title:	Assessment of Side Effects of Spirotetramat OD 150 on Honey Bee (*Apis mellifera* L.) Applied to Citrus in the Field in Spain Date: 2008-09-15
Organisation:	[redacted] Germany Bayer CropScience AG, [redacted], Germany
Report No.:	S08-00581; M-307363-01-1
Publication:	unpublished
Dates of experimental work:	March 20, 2008 - May 07, 2008
Guidelines:	OEPP/EPPO 170 (3) (2001)
Deviations:	No major deviations
GLP	Yes (certified laboratory)

Executive summary

This study aimed to determine potential effects of Spirotetramat OD 150 under realistic field conditions in a *Citrus* orchard to honey bee (*Apis mellifera*) colonies in Spain. Particular attention was directed to the development of the bee brood and potential brood effects.

The study comprised one trial which was carried out in *Citrus* sp. in Spain, consisting of one test item treated orchard (5297 m^2; 2 application dates) and an untreated control orchard (5328 m^2). The test orchards were isolated and not close to other flowering crops or extensive blooming weeds, which might have been attractive to bees. In the test item orchard (T) Spirotetramat OD 150 was applied to the crop once before flowering and once during flowering of the crop. The applications were carried out in a 3 weeks interval at an application rate each of 192.0 g a.s./ha (equivalent to 96.0 g a.s./ha/m canopy height - considering an average crown height of the orchard of approx. 2 m) in 800 L water/ha/m crown height. An orchard of untreated *Citrus* sp. was used as control group.

Six commercial bee colonies were placed in both orchards, respectively, before the 2nd application in the test item orchard (T) as soon as enough flowers were present to allow foraging of the bees. Each hive contained a healthy and queen-right bee colony containing one body with 12 frames and approximately 5,145 to 9,636 bees per colony. Colonies comprised at least 5 brood combs with all brood stages and a least two honey and pollen combs. Bees were free of *Nosema* and *Varroa* disease symptoms

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and other bee diseases. Hives were set up seven days before the second application in the test item orchard T, to let bees become familiar with the environment and to stabilise the increased mortality due to the transport. The condition of all colonies was checked once before set-up of the hives in the test orchards and four times afterwards at weekly intervals during flight activity of the bees in the orchard (DAA2 (days after 2nd application) +7, +14, +20, +26). During each assessment the bee colonies were observed for potential treatment-related effects on brood (eggs, unsealed brood – larvae, and sealed brood - pupae), as well as hive weight, colony strength, pollen and nectar/honey storage.
Additionally, the number of foraging bees as well as mortality in front of each hive and on three impartially selected places in each test orchard were assessed during the study. Mortality of the bees was checked during 3 days prior to the setup of the hives in the orchards, and mortality and foraging activity after set-up of the hives in the orchards during 6 days prior to the 2nd application in the test orchard and followed up during 26 days after the second application.
In addition samples of flowers, nectar and pollen were collected for residue analysis of spirotetramat (BYI 08330 and its enol-metabolite). Flowers were collected at three dates during flowering period – at start of flowering (between 1st and 2nd application), at full flowering and end of flowering (both after 2nd application). An additional sampling was done 26 days after the 2nd application since flowers were still available. Pollen (for residue analysis and pollen source identification) and nectar (residue analysis) samples from combs were taken at three dates during the exposure in the orchards. Samples were extracted during the brood assessment on DAA2 +7, DAA2 +14 and DAA2 +26.
The test item treatment did not result in an adverse effect on honey bees as determined by mortality and flight intensity. Differences of bee behaviour between control and treatment group were not observed. The condition of the colonies as assessed by colony strength and size of the brood nest was not affected by the treatment. No evidence of an irritation or termination of the development based upon exposure to treated crops was obtained in the colonies of the test item group and the control colonies.

MATERIAL AND METHODS

A Materials

1. Test material	Spirotetramat OD 150B G
Description	beige suspension
Lot/batch No.	batch No.: 2007-011635,
Content a.s.	149.0 g/L (analysed)
Stability of test compound	Approved until 20010-03-17 when stored at room temperature (25 ± 5°C)
2. Vehicle and/or positive control	Water
3. Test animals	
Species	The test species was *Apis mellifera mellifera* L. Six normally developed, healthy and queen-right bee colonies were used per orchard (12 in total). Each colony contained one body with 12 frames and approximately 5,145 to 9,636 adult bees per colony at test initiation (except colony C4 which contained over 13,813 bees). At this period of the year the hives may differ strongly in number of bees per hive, number of brood frames and hive weight. The hives were distributed according to number of brood frames and hive weight at start of the study and not selected for further criteria to reflect the normal apicultural situation in Spain. The hives contained at least 5 combs for broods of all ages and 2 honey and pollen combs. Bees were free of *Nosema* and *Varroa* disease symptoms and other bee diseases.

B Study design and methods

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1. In life dates March 20, 2008 - May 07, 2008
2. Experimental treatments

   The study comprised one trial which was carried out in *Citrus* sp. in Spain in the region of Valencia, consisting of one test item treated orchard (2 application dates) and an untreated control orchard. In the test item orchard (T) Spirotetramat OD 150 was applied to the crop once before flowering and once during flowering of the crop. The applications were carried out in a 3 weeks interval at an application rate each of 192.0 g a.s./ha (equivalent to 96.0 g a.s./ha/m canopy height - simulating an average crown height of the orchard of approx. 2 m) in 800 L water/ha/m crown height.

   The test item Spirotetramat OD 150 was applied once three weeks before flowering and once during flowering of the crop. The applications were carried out in a 3 weeks interval at an application rate each of 192.0 g a.s./ha (equivalent to 96.0 g a.s./ha/m canopy height - considering an average crown height of the orchard of approx. 2 m) in 800 L water/ha/m crown height. An orchard of untreated *Citrus* sp. was used as control group.

   Six commercial bee colonies were placed in the middle of the orchards, respectively, before the 2nd application in the test item orchard (T) as soon as enough flowers were present to allow foraging of the bees. The colonies were transported away from the treated orchard T and from the control orchard 35 days after the 2nd application at end of flowering period. The colonies were moved from the test orchards to an area where no flowering main crops were in the near surroundings. Control and treatment group colonies were set up together there.
3. Observations

   Mortality

   In order to record the number of dead honey bees in the treatment and control colonies, water-permeable linen sheets of 2.0 m width and about 9.0 m length were spread out in front of the six hives per treatment. Dead bee traps were attached to the entrance of the hives in order to register those dead bees which were carried out of the hives.

   Furthermore, the mortality was recorded at three impartially selected places in each test orchard. Therefore, linen sheets (covered area: approximately 36 m²) were spread out on three places in the orchard prior to the set-up of the hives for counting the number of dead bees on the sheets.

   The dead honey bees were differentiated in adult worker bees, drones, freshly emerged bees, pupae and larvae during each assessment and recorded in the field book.

   Three days before set-up of the hives evaluation of mortality was performed once a day (dead bee traps only). From set-up until one day before and from one day until 7 days after the second application, assessment of the mortality was performed once a day (dead bee traps and linen sheets). Subsequently, evaluations were performed every second day until the end of exposure. On the day of the second application, mortality assessment was performed 3 times during the day (morning, midday, evening).

   Foraging Activity and Flight Intensity of the Bees in the Field

   The observations of the flight intensity and foraging activity in the field, which started one day after the set-up of the colonies, took place at five locations each comprising approx. 25 flowers per location distributed uniformly over the test and control plots. The locations were located at 5 different trees and approx. 25 completely open flowers were chosen for observation per location. The locations changed during the exposure period due to flowering stage of the blossoms. At each assessment date the number of bees that were either foraging on the marked flowers or flying over the flowers was observed for one minute. Assessments were performed once a day starting from set-up of the hives in the fields until 7 days after the second application. Afterwards, assessments were performed every second day. On the day of application assessments were performed three times a day (morning, midday and evening).

   Brood Development:

The condition of all colonies was checked once before set-up of the hives in the test orchards (between 1st and 2nd application) and four times afterwards during flight activity of the bees in the test orchards (7, 14, 20 and 26 days after the 2nd application). At each brood evaluation the hives were weighed to assess honey collection and the increase of hive weight.
In order to record effects of the test item treatment compared to the control, the following parameters were assessed:

- Colony strength by estimating the number of bees (estimation according to Imdorf & Gerig, 1999, and Imdorf et al., 1987, see details below)
- Number of combs containing brood
- Presence of a healthy queen (e.g. by freshly laid eggs)
- Visual assessment of the pollen storage area and area with nectar (in %, see details below)
- Visual assessment of the area containing cells with eggs, larvae and capped cells (in %, see details below)

At each assessment the total of both sides of one comb were assigned to be 100% and the percentage area containing the brood stages, pollen and nectar on the comb was estimated. This was done for all combs per hive. Afterwards the mean values were calculated for each hive and assessment date.
For the determination of the strength of the colonies during the brood evaluations each side per comb was divided in 8 squares, each square which is completely covered by bees being equivalent to 125 bees. The number of bees was classified in a scheme numbering from 1 to 8, where 1 is equivalent to 125 bees and 8 to 1000 bees. Bees sitting on both sides of each frame and on the walls of the hives were classified, added together and multiplied by 125 to estimate the number of bees present in the hives.

Observation at the Entrance of the Hives

Additionally to the assessments of mortality, flight intensity and brood development, the behaviour of the bees on the crop and around the hive was observed on the days of bee-exposure in the orchards. This was done at the same time as the flight intensity assessments in the test orchards.

Collection of Flowers for Residue Analysis

Flowers were collected at three dates during flowering period – at start of flowering (between 1st and 2nd application), at full flowering and end of flowering (both after 2nd application). An additional sampling was done 26 days after the 2nd application since flowers were still available. Each sample was taken from at least 12 trees randomly distributed over the orchard and containing at least 5 g. Per test orchard five independently samples were taken.

Collection of Pollen and Nectar Residue Analysis and Pollen for Pollen Source Identification

Pollen and nectar samples from combs were taken at three dates during the exposure in the orchards. Samples were extracted during the brood assessment on DAA2 +7, DAA2 +14 and DAA2 +26 from each hive separately containing at least 1 g (residue analysis). The amount of pollen for the pollen source identification was partly reduced at the first sampling date due to low pollen production of the *Citrus* flowers.

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RESULTS AND DISCUSSION

A. Findings:

Toxicity to Honey Bees, Field Test

Test item		Spirotetramat OD 150	
Test object		*Apis mellifera*	
Exposure		Two applications of Spirotetramat OD 150 three weeks before start of full flowering of the crop and at beginning of full flowering during foraging activity	
Treatment group		**Test item treatment**	**Control**
1st application date (20 Mar 2008, before flowering): application rate g a.s./ha		192.0*	-
2nd application date (11 Apr 2008, start of full flowering): application rate g a.s./ha		192.0*	-
Spray volume per ha [L water/ha/m crown height]		800	-
Mean mortality [dead bees/colony/ assessment day]	Pre-set-up [DAA2 -9 to -7]:	24.4	35.1
	Pre-appl. [DAA2 -6 to 0ba]:	9.2	19.0
	Pre-appl. [DAA2 0ba]:	9.3	12.8
	Post-appl. [DAA2 0aa]:	4.8	3.7
	Post-appl. [DAA2 0aa to 26]:	14.9	18.1
Mean number dead pupae/larvae per colony/assessment day (post-application, DAA2 0aa to 26)		0.18	0.26
Mean flight intensity [foraging bees/minute/ 25 flowers]	Pre-appl. [DAA2 -6 to 0ba]:	2.5	2.7
	Pre-appl. [DAA2 0ba]:	1.6	3.6
	Post-appl. [DAA2 0aa]:	1.3	2.0
	Post-appl. [DAA2 0aa to 26]:	2.0	2.0

0ba = before second application
0aa = after second application
DAA2 = days after second application
* = equivalent to 96.0 g a.i./ha/m canopy height - simulating an average crown height of the orchard of approx. 2 m

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Analytical findings in *Citrus* Flowers, Pollen and Nectar

Sample Type	Applications	Application rate [g a.s./ha/m CH]	Canopy height [m]	Date of sampling	Days after 2nd application	Residue of spirotetramat and BYI 08330-enol Mean (minimum – maximum) [mg/kg] per sampling date	per sample Type
Flower	1st appl.: pre-blossom (2008-03-20) 2nd appl.: full flowering (2008-04-11)	96 96	2.0	2008-04-08 2008-04-18 2008-04-25 2008-05-07	-3 +7 +14 +26	0.11 (0.07 – 0.16) 0.38 (0.13 – 0.98) 0.24 (0.07 – 0.43) 0.11 (0.08 – 0.14)	0.21 (0.07 – 0.98)
Pollen				2008-04-18 2008-04-25 2008-05-07	+7 +14 +26	0.03 (0.02 – 0.06) 0.07 (0.05 – 0.16) 0.09 (0.03 – 0.17)	0.06 (0.02 – 0.17)
Nectar				2008-04-18 2008-04-25 2008-05-07	+7 +14 +26	< LOQ < LOQ < LOQ	< LOQ

LOQ $_{spirotetramat}$: 0.01 mg/kg
LOQ $_{spirotetramat-enol}$: 0.01 mg/kg
CH: Canopy Height

B. Observations

Honey bee mortality

During pre-application period, the mortality was 35.1 dead bees/colony/day in the control group (C) and 24.4 dead bees/colony/day in the test item treated group (T) before set-up of the hives in the orchards. After set-up, but before the second application, mortality was 19.0 dead bees/colony/day in C and 9.2 dead bees/colony/day in T. At the assessments after the second application during exposure (DAA2 0: day of 2nd application) the mean mortality in all treatment groups was on a low level and no test item related increase in the number of dead bees was observed (T: 4.8 dead bees/colony/day, control: 3.7 dead bees/colony/day). No test item related difference in the number of dead bees of the treatment groups T compared to the mortality in the control group was observed at any time after the applications. The daily mean post-application mortality (DAA2 0 - 26) in the test item treatment group T was 14.9 dead bees/colony/day and 18.1 dead bees/colony/day in the control group.
The mean number of dead pupae and larvae from day 0 to 26 after the second application during exposure was on a low level in both groups with 0.18 dead pupae and larvae/colony/day in the treatment group T and 0.26 dead pupae and larvae/colony/day in the control group. In so far, larval and pupae mortality was at a comparable low level in both groups, treatment-related effects were not observed.

Honey bee flight intensity

The daily mean flight intensity (forager bees/minute/25 flowers) before the second application during exposure was 2.5 in the test item treatment group T and 2.7 in the control group C. The mean flight intensity after the applications on DAA 0 was 1.3 forager bees/minute /25 flowers in the treatment group T and 2.0 forager bees/minute/25 flowers in the control group. The daily mean post-application (DAA2 0aa to DAA2 26) flight intensity was 2.0 forager bees/minute/25 flowers in both groups.

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Conditions of the colonies and honey bee brood development

The mean strength of the colonies (mean number of bees) in the test item treatment group and in the control group was 6,938.2 and 9,065.9 bees per hive at the brood assessment before start of exposure. On the last assessment 26 days after the second application the mean strength of the colonies ranges from 12,300 to 12,200 bees per hive in the test item group and in the control group, respectively. The brood nest size changed only slightly during the observation period, so there was no test item related difference in the development of the brood nest.

On the frequent assessments during exposure in the test orchards, all colonies in both treatment groups had all brood stages and similar development. Only one colony in the control treatment showed a lack of eggs at the last assessment date.

However, all brood stages in all other colonies of the groups were available at the different assessment dates during the experimental phase of the study which shows that the colonies and the queens were in good condition during the observation period.

Before start of exposure the mean percentage of eggs, larvae and pupae per hive was 3.6, 6.4 and 14.2% in the test item treated hives, and 3.8, 6.4 and 14.7% in the control hives, respectively, with an increase at start of exposure due to full flowering stage of the citrus trees and with a decrease at end of flowering period. At the last assessment the mean percentage of eggs, larvae and pupae per hives was 2.2, 5.1 and 13.5% in the test item treated hives and 1.5, 6.0 and 11.7% in the control hives with no differences between either group. Before start of exposure 24.2, 29.8 and 46.0% of the hive area was covered by brood, food and empty cells, respectively, in the test item treated hives, and 24.9, 29.0 and 46.1% in the control hives. At the end of exposure 20.9, 46.6 and 32.5% of the hive area was covered by brood, food and empty cells, respectively, in the test item treated hives, and 19.3, 39.8 and 40.9% in the control hives.

Honey bee behaviour in front of the colonies and within the crop

No differences regarding the behaviour of the bees were observed between the test item treatment group T and the control group C.

Pollen Source Identification

At three dates (DAA2 +7, DAA2 +14 and DAA2 +26) during the exposure of the bee hives in the test orchards samples for pollen source identification were collected.

The bees which were foraging in the control orchard collected mainly pollen of different wild flowers (ranging from 36.8% on DAA2 +26 to 39.7% on DAA2+7), *Citrus* (from 8.7% on DAA2 + 26 to 23.7% on DAA2+14), *Quercus ilex* (between 2.3% on DAA2 +26 and 17.2% on DAA2 +7%) and *Hypecou*m sp. (from 9.8% on DAA2 + 14 to 17.5% on DAA2 +7). At the end of the exposure period 52.7% of *Olive* tree pollen was found in the pollen storage.

In the test item treated orchard little *Citrus* pollen was collected (ranging from 1.0% on DAA2 +26 to 2.7% on DAA2 +7). Mainly *Helianthemum* sp. (between 24.5% on DAA2 +26 and 61.0% on DAA2 +7), *Quercus ilex* (between 2.7% on DAA2 +7 and 45.7% on DAA2 +26) and pollen of different wild flowers (between 13.5% on DAA2 +26 and 30.2% on DAA2 +7) were found in the pollen storage of the hives[12].

[12] Although the proportion of citrus pollen among the total pollen collected by the bees is low (in particular in the treatment replicate), exposure of the bees to the treatment can nevertheless be considered to be sufficient and representative under natural conditions: on one hand, citrus is intrinsically not a very attractive pollen source for bees, and on the other hand, the assessment of foraging intensity (see above) demonstrates that sufficient foraging activity in the target crop was given, and that foraging in control and treatment was at the same level during the exposure period. Therefore, it can be concluded that the bees were sufficiently exposed in the control as well as in the treatment group, in spite of the low quantity of citrus pollen found in the hives of the treatment replicate.

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Residue analysis

Analysis of residues of spirotetramat (BYI 08330 and its enol metabolite) in the flowers, nectar and pollen were carried out. Samples were collected at three dates (four dates for flower collection) during the flowering period – once at start of flowering (between 1st and 2nd application), and two times (three times for flower collection) at full flowering until end of flowering (after 2nd application).
No residues at or above the respective LOQ level were found in any of the control samples.
In treated flower samples, the residues of spirotetramat (BYI 08330) ranged from 0.02 to 0.52 mg/kg. The residues of BYI08330-enol ranged from 0.04 to 0.46 mg/kg, with the highest amount of residues found on DAA2 +7, decreasing until end of exposure.
In treated pollen samples, the residues of spirotetramat ranged from < 0.01 to 0.03 mg/kg. The residues of BYI08330-enol ranged from < 0.01 to 0.15 mg/kg.
In treated nectar samples, no residues of spirotetramat or BYI08330-enol at or above the LOQ level of 0.01 mg/kg were found.

CONCLUSION

The test item treatment did not result in an adverse effect on honey bee as determined by mortality and flight intensity. Differences of bee behaviour between control and treatment groups were not observed. The condition of the colonies as assessed by colony strength, size of the brood nest and brood development was not affected by any of the treatments. No evidence of an irritation or termination of the brood development based upon exposure to treated crops was obtained in the colonies of the test item group and the control colonies indicating that the test item treatment did not provoke any effects on any of the bee brood stages.

([redacted] S., 2008)

Report:	**KIIIA1 10.4.5/05, [redacted] T., [redacted] J., [redacted] C., [redacted] H.-J., [redacted] R., [redacted], M.T.; 2010**
Title:	Assessment of effects of Spirotetramat OD 150 to honey bees (*Apis mellifera*) and their colonies in the field in a citrus crop – GLP trial 2009 Date: 2010-02-17
Organisation:	Bayer CropScience AG, [redacted], Germany
Report No:	MAUS/AM050; M-363607-01-3
Publication:	Unpublished
Dates of experimental work:	2009-07-23 to 2010-01-22
Guidelines:	EPPO 170 (3), US EPA OPPTS 850.3040 (SUPP)
Deviations	None
GLP:	yes (certified laboratory)

Executive summary
The aim of the study was to determine potential effects of Spirotetramat OD 150 under realistic field conditions in citrus to honey bee (*Apis mellifera*) colonies. A special focus was made on potential brood effects.
There were 2 treatment groups (12 colonies per treatment group) with treatment group 1 as untreated control. Treatment group 2 received 2 applications with 96 g a.s./ha in canopy height in a spray interval of 14 days or 16 days. Mortality was assessed for up to 6 days in 24 hour intervals and thereafter in 48 hour intervals after the applications (except for dates, when T0 to T3 assessments were performed, or in case of unfavourable weather conditions). Furthermore the number of citrus blossoms on 2 randomly chosen branches on 5 citrus trees and the number of foraging bees on the citrus blossoms were assessed. No treatment-related effect on brood development and abundance of adult bees was found. No effects on foraging activity, the storage behaviour of the honey bees, the hive weight development and mortality were found.

Material and Methods

A Materials

1. Test material	Spirotetramat OD 150
Description	beige suspension
Lot/batch No.:	Batch ID: 2009-003718 TOX No.: 08652-00
Analytical content a.s.	151.2 g/L, (15.3 %)
Stability of test compound	Expiry date: 2011-05-28, when stored at +2 °C to + 30 °C
2. Vehicle and/or positive control	--
3. Test animals	
Species	Honey bee (*Apis mellifera* L.)
Age	Brood of all ages
Source	Honey bee colonies, homogenous regarding population, colony strength, food storage, brood status and preparation, disease-free and queen-right, bred by a professional beekeeper and queen breeder
Collection	--
Environmental conditions	Study plots (2-3 ha each) in the surroundings of Alem (province of Missiones, Argentina)
Temperature	14.2 – 34.3°C
Relative humidity	34 – 96.7%
Photoperiod	Length of the days

B Study design and methods

1. In life dates	July 23, 2009 - January 22, 2010

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2. Experimental treatments

There were 2 treatment groups, each with 4 replicates. Treatment group 1 (plots 1, 5, 6 and 7) served as untreated control. The plots of treatment group 2 received 2 applications in a spray interval of 14 days or 16 days. All plots were in approximately 3 km distance of each other.

Three bee hives were set up on each plot. Each hive contained a colony of approximately in average 33,000-40,000 bees (*Apis mellifera mellifera* L.) plus a queen of the same maternal origin (sister queens) at the start of the study and comprised of 3-4 frames for brood of all ages, 4 honey frames and 1 feeder. The hives were set up on the plots 5 weeks before the 1st application. Before performance of the 1st application, the 1st brood assessment T0 (pre-treatment assessment) was conducted. Thereafter the brood assessments T1 to T4 were performed in approximately weekly intervals. After the T1 assessment a second hive box (honey super) was set on top of the hives. The hives remained on the citrus plots until the last weekly in-field brood assessment T4 was conducted. Thereafter the colonies were transferred for further monitoring to an area of less intensive agriculture, where the last brood assessment T5 was conducted 2 weeks after hive relocation and approximately 4 weeks after the 2nd application had been conducted. Control colonies were assessed in parallel to the assessment dates of the respective treated groups. During the T_x assessments the bee colonies were observed for potential treatment-related effects on brood (eggs, unsealed brood - larvae, and sealed brood - pupae), as well as hive weight, colony strength, pollen and nectar/honey storage.

<u>Control</u>: The control plots remained untreated.

<u>Test item</u>: 2 applications with 96 g a.s./ha in canopy height in a spray interval of 14 days (plots 2 and 8) or 16 days (plots 3 and 4), resulting in total application rates of 278.4 g a.s./ha on plot 2, 240.0 g a.s./ha on plot 3, 230.4 g a.s./ha on plot 4, and 172.8 g a.s./ha on plot 8, respectively (differences in applications were based on canopy height).

<u>Application of the test item:</u>

The test item treatments were staggered in order to create work units, which can logistically and technically be handled in one day. The applications in treatment group 2 were performed on each plot by using a previously calibrated pneumatic electrostatic air blast sprayer of the company Martignani, series "Whirlwind-compact" B-612. During the application the hives and the water supply of the bees were covered with a plastic cover to prevent direct overspray with the test item. During the applications weather conditions were dry and the maximum wind speed was less than 10 km/h.

3. Observations

Mortality in front of each hive was assessed for up to 6 days in 24 h intervals and thereafter in 48 h intervals after the applications (except for dates, when T0 to T3 assessments were performed, or in case of unfavourable weather conditions). Furthermore (according to the same time table) the number of citrus blossoms was estimated using 2 randomly chosen branches of 5 impartially pre-selected citrus trees on each plot throughout the study. Concurrently, the number of foraging bees on the citrus blossoms was assessed.

Results and Discussion

A. Findings

The comb area containing brood of all stages (eggs, larvae and pupae) fluctuated in the control as well as in treatment group 2 (test item treatment) on the different assessment days, reflecting the typical natural variability of this endpoint. No treatment-related effect on brood and abundance of adult bees was found.

Foraging activity and hive weight development were unaffected in the control and the test item treatment. No effect on the storage behaviour of the honey bees regarding pollen and nectar/honey was found. No treatment-related effect was found on mortality of adult honey bees as well as on the number of dead larvae and pupae found in front of the bee hives.

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Table 1: Biological findings

Assessment	Parameter	Treatment group 1 (Control)				Treatment group 2 (Test Item Treatment - 2 x 96 g a.s./ha/m CH)			
		Plot No. 1	Plot No. 5	Plot No. 6	Plot No. 7	Plot No. 2	Plot No. 3	Plot No. 4	Plot No. 8
		% comb area per assessment as average of all hives per plot							
T0	**Egg cells**	4.4	4.4	6.3	4.6	5.4	5.4	3.9	7.7
T1		7.7	3.2	7.2	5.4	8.3	5.8	12.3	7.0
T2		9.1	7.3	6.2	5.0	8.5	7.7	9.4	11.1
T3		8.5	7.4	9.5	7.4	9.9	6.8	10.5	10.3
T4		10.6	5.9	4.6	10.2	8.9	9.4	5.6	10.6
T5		7.8	4.5	9.0	8.4	6.9	10.6	5.1	5.0
T0	**Unsealed brood**	16.0	20.6	20.5	14.9	24.4	16.6	13.7	19.0
T1		18.9	17.6	18.5	18.8	28.1	19.7	18.1	18.0
T2		19.1	14.4	15.6	19.4	21.5	19.9	22.3	11.5
T3		19.7	21.1	24.0	16.6	26.3	21.2	24.4	16.9
T4		15.9	21.6	21.8	19.5	27.2	19.8	25.4	19.1
T5		18.2	11.9	19.5	16.4	19.5	19.5	21.4	8.7
T0	**Sealed brood**	30.4	25.1	23.1	33.9	27.9	33.0	28.1	32.5
T1		28.8	33.7	32.7	38.8	25.9	33.6	32.0	35.4
T2		31.5	36.0	39.6	34.4	26.3	31.6	32.3	31.9
T3		31.8	32.2	36.0	32.0	27.3	31.5	30.6	30.6
T4		22.9	32.0	31.9	30.7	29.6	31.1	34.3	25.6
T5		26.3	29.6	24.8	29.4	26.8	22.9	27.6	24.9
T0	**Drone brood**	0.8	0.4	1.2	1.5	0.6	0.9	0.0	1.5
T1		1.7	0.6	3.6	1.2	1.5	1.4	1.6	1.9
T2		2.4	1.7	5.3	1.9	1.6	1.2	2.2	1.8
T3		1.3	2.1	4.6	2.0	1.3	1.2	2.8	2.0
T4		0.7	2.1	2.3	2.1	1.7	1.3	2.6	2.4
T5		1.6	0.8	3.0	1.7	0.7	0.6	1.7	2.0
T0	**Total brood**	51.6	50.4	51.1	54.9	58.2	55.8	45.7	60.6
T1		57.0	55.1	62.0	64.2	63.8	60.6	64.1	62.2
T2		62.0	59.4	66.7	60.6	57.9	60.4	66.3	56.2
T3		61.3	62.9	74.2	58.1	64.8	60.6	68.3	59.7
T4		50.1	61.7	60.6	62.6	67.4	61.7	67.8	57.8
T5		53.9	46.9	56.3	55.8	54.0	53.6	55.7	40.6
T0	**Comb area covered by adult bees (upper hive box)**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
T1		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
T2		16.0	14.7	8.9	22.5	7.4	22.9	0.6	12.7
T3		24.4	19.6	14.1	35.3	6.9	17.8	1.3	22.2
T4		22.0	36.5	30.4	33.8	37.2	38.0	11.9	49.2
T5		53.9	61.0	43.8	69.1	45.9	40.6	51.4	64.4

T1 – T5: assessment days
CH: canopy height
n.a.: not assessed
application dates in treatment group 2: at days of T0 and T2 assessment (performed before any application scheduled for that day)

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Table 2: Biological findings, continued

Assess-ment	Parameter	Treatment group 1 (Control)				Treatment group 2 (Test Item Treatment - 2 x 96 g a.s./ha/m CH)			
		Plot No. 1	Plot No. 5	Plot No. 6	Plot No. 7	Plot No. 2	Plot No. 3	Plot No. 4	Plot No. 8
		% comb area per assessment as average of all hives per plot							
T0	Comb area covered by adult bees (lower hive box)	69.6	73.3	73.3	62.0	73.3	74.1	75.3	75.7
T1		81.1	77.6	80.4	79.3	75.9	82.0	79.8	82.8
T2		80.9	82.4	83.7	83.9	80.6	82.2	80.7	86.9
T3		85.6	86.7	84.4	87.8	86.5	88.3	88.3	89.4
T4		84.8	78.3	78.0	80.6	78.3	84.4	86.3	82.8
T5		80.6	77.0	79.8	77.4	76.5	70.9	77.0	77.4
T0	Polen stores	4.1	7.7	9.8	6.8	4.7	7.6	8.6	6.0
T1		8.8	9.8	10.0	5.1	6.1	11.9	1.9	7.8
T2		9.3	11.2	10.6	7.3	9.6	14.4	9.1	0.2
T3		11.9	9.2	5.7	7.9	7.3	12.2	7.5	10.6
T4		16.5	6.9	8.2	6.3	6.1	13.5	5.8	10.6
T5		10.9	10.7	9.0	10.4	8.6	10.7	8.7	11.1
T0	Nectar/ honey stores	26.5	28.1	21.6	26.3	19.4	16.4	22.3	14.4
T1		27.4	25.8	16.9	22.2	21.9	17.5	18.2	21.9
T2		15.2	22.5	11.8	12.4	16.2	9.3	13.0	19.1
T3		13.1	16.5	6.8	8.3	6.9	5.3	7.6	6.4
T4		21.2	12.7	17.8	9.1	11.7	9.4	10.3	11.4
T5		28.5	36.5	25.1	27.0	28.5	23.9	26.1	38.9
T0	Empty cells	17.9	13.9	17.5	12.0	17.7	20.2	23.3	18.9
T1		6.8	9.3	11.1	8.5	8.2	10.1	15.7	8.1
T2		13.5	6.9	10.9	19.7	16.3	16.0	11.7	24.5
T3		13.8	11.5	13.3	25.7	21.0	21.9	16.6	23.3
T4		12.2	18.8	13.3	22.0	14.8	15.4	16.1	20.2
T5		6.6	5.8	9.6	6.8	8.9	11.8	9.4	9.4
		Estimated No. of adults [n] as average of all hives per plot							
T0	Estimated No. of adult honey bees (upper hive box)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
T1		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
T2		1,000	2,667	500	1,833	1,500	1,500	500	500
T3		1,500	1,000	1,833	2,000	333	1,333	500	2,333
T4		1,500	2,333	1,833	2,167	2,333	2,000	833	3,000
T5		4,000	4,000	3,333	6,333	4,167	3,667	4,333	3,667
T0	Estimated No. of adult honey bees (lower hive box)	33,333	36,667	33,333	40,000	35,667	33,333	35,000	40,000
T1		36,667	38,333	37,667	40,667	39,333	42,333	38,333	41,667
T2		42,333	38,667	40,000	45,000	40,000	44,000	40,000	43,333
T3		40,000	40,000	40,000	43,333	40,000	40,000	40,000	43,333
T4		41,667	40,000	43,333	45,000	41,667	42,667	43,333	45,000
T5		43,333	43,667	42,667	44,333	44,000	43,000	43,333	43,333
		Hive weight development [kg] as average of all hives per plot							
T0	Hive weight	20.5	21.4	20.6	23.1	20.8	21.0	20.3	21.4
T1		22.2	23.2	21.2	23.9	22.1	22.6	20.7	23.4
T2		20.9	22.5	21.1	23.3	21.0	21.9	19.9	22.3
T3		21.3	21.5	20.2	22.5	19.7	21.0	19.8	20.5
T4		22.9	22.5	22.6	25.2	20.6	24.8	23.1	23.3
T5		31.8	32.1	27.9	35.0	28.0	29.2	30.4	30.3

T1 – T5: assessment days
CH: canopy height n.a.: not assessed
application dates in treatment group 2: at days of T0 and T2 assessment (performed before any application scheduled for that day)

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Table 3: Biological findings, continued

Assessment	Parameter	Treatment group 1 (Control) Plot No. 1	Plot No. 5	Plot No. 6	Plot No. 7	Treatment group 2 (Test Item Treatment - 2 x 96 g a.s./ha/m CH) Plot No. 2	Plot No. 3	Plot No. 4	Plot No. 8
		Average No. of bees on the citrus blossoms per plot [n/min/assessment area]							
Ø after 1st to before 2nd appl.	Foraging bees on citrus blossoms	2.3	2.2	3.5	4.6	9.0	16.7	13.9	11.8
Ø after 2nd appl.	Foraging bees on citrus blossoms	25.4	8.4	19.8	29.6	13.2	4.3	7.8	5.8
		No. as total of all hives per plot [n]							
Σ after 1st to before 2nd appl.	Dead worker bees in front of hive	49	122	78	147	350	240	149	120
Σ after 2nd appl.		342	394	262	626	408	568*	324	484*
Σ after all appl.		391	516	340	773	758	808*	473	604*
Σ after 1st to before 2nd appl.	Dead drones in front of hive	1	1	7	2	5	16	7	8
Σ after 2nd appl.		18	21	31	28	17	65	28	19
Σ after all appl.		19	22	38	30	22	81	35	27
Σ after 1st to before 2nd appl.	Dead pupae in front of hive	1	12	3	2	23	12	22	13
Σ after 2nd appl.		24	24	13	28	23	14	11	13
Σ after all appl.		25	36	16	30	46	26	33	26
Σ after 1st to before 2nd appl.	Dead larvae in front of hive	1	0	0	1	0	0	1	0
Σ after 2nd appl.		0	2	0	0	8	2	0	2
Σ after all appl.		1	2	0	1	8	2	1	2
Σ after 1st to before 2nd appl.	Mal-formed in front of hive	0	0	0	0	0	0	0	0
Σ after 2nd appl.		0	1	1	0	0	1	0	1
Σ after all appl.		0	1	1	0	0	1	0	1

CH: canopy height
*Remark: The increased number of dead honey bees on plot 3 and 8 caused by several robbery events by feral honey bees were excluded from the calculation.

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Residue analysis:

Mean residues of spirotetramat (BYI 08330) and its metabolites BYI08330-enol, BYI08330-mono-hydroxy, BYI08330-ketohydroxy and BYI08330-enol-glucoside in/on citrus flowers after the spray applications to citrus plants:

Table 4: Summary: Findings of residue analysis

Treat-ment group	Time	DALT	Residues of BYI 08330 [mg/kg]	Residues of BYI 08330 cis-enol * [mg/kg]	Residues of BYI 08330 mono-hydroxy * [mg/kg]	Residues of BYI 08330 cis-keto-hydroxy * [mg/kg]	Residues of BYI 08330 enol-glucoside* [mg/kg]	Total Residues of BYI 08330 [mg/kg]
			Mean	Mean	Mean	Mean	Mean	Mean
Control	After 1st appl.	1	< 0.01	< 0.012	< 0.012	< 0.012	< 0.007	< 0.053
		7	< 0.01	< 0.012	< 0.012	< 0.012	< 0.007	< 0.053
		15	< 0.01	< 0.012	< 0.012	< 0.012	< 0.007	< 0.053
	After 2nd appl.	1	< 0.01	< 0.012	< 0.012	< 0.012	< 0.007	< 0.053
		7	< 0.01–0.01	< 0.012	< 0.012	< 0.012	< 0.007	< 0.053-0.053
		14/15	< 0.01	< 0.012	< 0.012	< 0.012	< 0.007	< 0.053
Test item Treat-ment	After 1st appl.	1	0.81 - 3.2	0.88 - 3.7	< 0.012	0.02 - 0.07	<0.007-0.02	1.7 - 7.0
		7	0.04 - 0.08	0.32 - 2.0	< 0.012	0.05 - 0.25	0.02 - 0.10	0.44 - 2.4
		15	<0.01 - 0.02	0.10 - 0.70	< 0.012	0.02 - 0.16	0.01 - 0.11	0.16 - 0.99
	After 2nd appl.	1	3.8 - 11.0	6.8 - 11.0	< 0.012	0.18 - 0.46	0.05 - 0.08	13.0 - 22.0
		7	0.05 - 0.10	0.98 - 1.7	< 0.012	0.15 - 0.30	0.05 - 0.13	1.2 - 2.3
		14-16	0.01 - 0.03	0.20 - 0.54	< 0.012	0.06 - 0.23	0.05 - 0.14	0.34 - 0.90

DALT : Days after Application
appl. application
*: Residues are given as Parent Equivalent

B. Observations

The comb area containing brood of all stages (eggs, larvae and pupae) fluctuated in the control as well as in treatment group 2 (test item treatment) on the different assessment days, reflecting the typical natural variability of this endpoint. No treatment-related effect on brood and abundance of adult bees was found.

Foraging activity and hive weight development were unaffected in the control and the test item treatment. No effect on the storage behaviour of the honey bees regarding pollen and nectar/honey was found. No treatment-related effect was found on mortality of adult honey bees as well as on the number of dead larvae and pupae found in front of the bee hives.

CONCLUSION

No effect on brood development (eggs, larvae, pupae) and abundance of adult honey bees was found after the application of 2 x 96 g spirotetramat/ha/m canopy height (total application rates from 172.8 g a.s./ha to 278.4 g a.s./ha, depending on respective tree height; spray interval of 14-16 days) in flowering citrus. Likewise, no effects on foraging activity, mortality determined in front of the hives, hive weight development and the food storage behaviour of honey bee colonies were found.

([redacted] *et al.*, 2009)

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Report:	**KIIIA1 10.4.5/06, [redacted] R. E. L., [redacted] G. R.; 2010**
Title:	Field investigation of exposure and effects of Movento® to honey bees from application to citrus during bloom Date: 2010-07-16
Organisation:	[redacted], NC, USA Bayer CropScience AG, [redacted], Germany
Report No:	EBFNP158; M-386205-01-1
Publication:	Unpublished
Dates of experimental work:	In-citrus phase: 2009-03-22 to 2009-04-16 Migratory phase: 2009-04-18 to 2009-10-26
Guidelines:	None. Study based upon OPPTS Draft Guideline 850.3040, Field Testing for Pollinators
Deviations:	Not specified
GLP:	No

Executive summary

The aim of the study was to investigate the potential effects of Spirotetramat SC 240 G (Movento®) to honey bees (*Apis mellifera* L.) in colony feeding tests.

Apis mellifera bees (12 hives of bees at one citrus grove) were exposed to Spirotetramat SC 240 G at a maximum labelled rate of 731 mL/ha (equivalent to 175 g a.s./ha) via application to citrus at the beginning of the bloom period and then the hives were monitored for the remainder of bloom. A control group (12 hives of bees at one citrus grove) received no treatment. Colonies were assessed for strength and health (i.e., adult and brood populations, food stores, pests and diseases) and brood effects. Pollen, nectar, honey and wax samples were analyzed for presence of residues of spirotetramat.

Quantifiable residues of total residues of spirotetramat and its metabolite spirotetramat enol were detected in pollen and nectar for only a few weeks post-application to blooming citrus. No quantifiable residues were present in samples of stored pollen and honey or in the citrus blossoms collected the following year.

There were no significant differences between the control and treatment groups of hives for any colony strength and health measurements and brood cohort success during the in-citrus phase.

Based on the results of this study, it appears that Movento® has a high margin of safety to honey bees when it is applied to citrus during bloom. Residue levels in pollen and nectar were well below levels of possible toxicological concern for both adult bees and bee brood.

MATERIAL AND METHODS

A Materials

1. Test material	Spirotetramat SC 240 G
Description	not specified
Lot/batch No.	US EPA Registration No.: 264-1050
Analytical content a.s.	22.4 %
Stability of test compound	not specified
2. Vehicle and/or positive control	<u>Vehicle</u>: Field test: surfactant CNI 800 ([redacted], GA, USA) was added at a rate of 250 mL/ 100 mL water.
3. Test animals	
Species	Honey bee (*Apis mellifera* L.)
Age	colonies including immature and adult stages
Source	Honey bee colonies supplied by a migratory beekeeper
Collection	--
Environmental conditions	Treated citrus grove (approx. 5 ha) north of [redacted], Florida, USA;

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	control citrus grove (15 ha) north of [redacted]; Florida, USA
Temperature	37.2 – 91.8°F (approx. 90°F)
Relative humidity	not specified
Photoperiod	Length of the days

B Study design and methods

1. In-citrus phase	March 22, 2009 - April 16, 2009
Migratory phase	April 18, 2009 - October 26, 2009

2. Experimental treatments

Three replicates of 4 hives of honeybees (*Apis mellifera*) were placed at the edge of each of two citrus groves at start of bloom and removed after bloom finished. One grove (approx. 5 ha) received a single application of Spirotetramat SC 240 at full citrus label rate.

Control: The other grove (15 ha) received no treatments (control).

Test item: Maximum labelled rate of the test item in the colony feeding test was 731 mL product/ha (equivalent to 175 g a.s./ha).

Application of the test item in the contact test:

Spirotetramat SC 240 G was applied by a tractor pulled orchard air-blast sprayer (Tank volume: 3785 L, output: 1870 L/ha, travel speed: 4.7 km/h) once at early blossom in citrus at the maximum labelled rate. The surfactant CNI 800 ([redacted], GA, USA) was added at a rate of 250 mL/ 100 mL water.

3. Observations

The colony strength and health, the intra-hive mortality, brood development, hive weight change, spirotatramat residues and bee behaviour were measured. The first assessment was conducted prior to study initiation in citrus and the second assessment was conducted in April after citrus bloom and prior to hives being moved out. The last assessments were carried out in May after apple pollination, in June after blueberry pollination and in October after late summer pumpkin pollination.

The software used to perform the statistical analysis were Microsoft Excel analysis tools and XLStat Pro.

RESULTS AND DISCUSSION

A. Findings

Quantifiable residues of total Spirotetramat SC 240 G residues (spirotetramat and the metabolite, spirotetramat enol) were detected in bee-collected pollen and nectar for only a few weeks post-application to blooming citrus. Highest individual detects were 3.32 ppm in whole blossoms, 0.55 ppm in bee-collected pollen and 0.04 ppm in bee-collected nectar, see also tables below.

Table 1: Mean Movento residues in whole citrus blossoms.
LOQ = 0.01 ppm parent spirotetramat and -enol

Date	DAA	Treatment	n	Mean Parent (ppm)	Mean Enol metabolite (ppm) [1]	Mean Total Residue (ppm)	Total residue range (ppm)
Whole Citrus Blossoms							
25 Mar 2009	-1	Movento	1	<0.01	<0.01	<0.01	a
		Control	1	<0.01	<0.01	<0.01	
27 Mar 2009	+1	Movento	1	1.74	1.58	3.32	
		Control	1	<0.01	<0.01	<0.01	
02 Apr 2009	+7	Movento	1	0.05	0.31	0.36	
		Control	1	NA	NA	NA	
05 Apr 2010	+375	Movento	4	<0.01	<0.01	<0.01	<0.01
		Control	0	NA	NA	NA	NA

1 = enol metabolite is presented as the parent equivalent. The parent equivalent calculation = enol measured concentration x 1.36.
a = there was only one composite sample of blooms; therefore a range is not applicable for these intervals.
NA = no sample available or analyzed

Table 2: Mean Movento residues in samples of bee-collected pollen and bee-collected nectar.
LOQ = 0.01 ppm parent spirotetramat and -enol

Date	DAA	Treatment	n	Mean Parent (ppm)	Mean Enol metabolite (ppm) [1]	Mean Total Residue (ppm)	Total residue range (ppm)
Bee-collected Pollen [2]							
26 Mar 2009	-1	Movento	5	<0.01	<0.01	<0.01	<0.01
		Control	5 a	0.01	0.01	0.02	<0.01 - 0.09
28 Mar 2009	+1	Movento	6	0.11	0.06	0.17	0.05 - 0.31
		Control	0	NA	NA	NA	NA
31 Mar 2009	+4	Movento	6	0.04	0.13	0.17	0.07 – 0.32
		Control	1	<0.01	<0.01	<0.01	<0.01
03 Apr 2009	+7	Movento	6	0.05	0.06	0.11	0.04 – 0.23
		Control	6	<0.01	<0.01	<0.01	<0.01
18 Apr 2009	+23	Movento	3	<0.01	<0.01	<0.01	<0.01
		Control	3	<0.01	<0.01	<0.01	<0.01
Bee-collected nectar							
25 Mar 2009	-1	Movento	12	<0.01	<0.01	<0.01	<0.01
		Control	12	<0.01	<0.01	<0.01	<0.01
27 Mar 2009	+1	Movento	12	<0.01	0.01	0.01	<0.01 – 0.04
		Control	12	<0.01	<0.01	<0.01	<0.01
30 Mar 2009	+4	Movento	6	<0.01	0.02	0.02	0.02 – 0.03
		Control	0	NA	NA	NA	NA
02 Apr 2009	+7	Movento	6	<0.01	0.01	0.01	0.01 – 0.03
		Control	1	<0.01	<0.01	<0.01	<0.01

1 = enol metabolite is presented as the parent equivalent. The parent equivalent calculation = enol measured concentration x 1.36.
2 = bee-collected pollen is also referred to as trapped pollen in this report.
NA = no sample available or analyzed
a = Detection of spirotetramat residues in one control pollen sample almost certainly represents contamination (source unknown, but may have been inadvertent contamination – see file note).
Range in all control samples on -1 DAA was <0.01 – 0.09 ppm

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Table 3: Mean Movento residues in samples of stored pollen and capped honey from comb. LOQ = 0.01 ppm parent spirotetramat and -enol

Date	DAA	Treatment	n	Mean Parent (ppm)	Mean Enol metabolite (ppm) [1]	Mean Total Residue (ppm)	Total residue range (ppm)
Capped Honey							
15-18 Apr 2009	+20-23	Movento	12	<0.01	0.02	0.02	0.01 – 0.04
		Control	12	<0.01	<0.01	<0.01	<0.01
11-15 May 2009	+46-50	Movento	6	<0.01	<0.01	<0.01	<0.01
		Control	6	<0.01	<0.01	<0.01	<0.01
16-18 Jun 2009	+82-84	Movento	6	<0.01	<0.01	<0.01	<0.01
		Control	4	<0.01	<0.01	<0.01	<0.01
23-25 Oct 2010	+211-213	Movento	5	<0.01	<0.01	<0.01	<0.01
		Control	3	<0.01	<0.01	<0.01	<0.01
Stored Pollen [2]							
15-18 Apr 2009	+20-23	Movento	12	0.03	0.13	0.14	<0.01 – 0.55
		Control	12	<0.01	<0.01	<0.01	<0.01
11-15 May 2009	+46-50	Movento	6	<0.01	<0.01	<0.01	<0.01
		Control	6	<0.01	<0.01	<0.01	<0.01
16-18 Jun 2009	+82-84	Movento	6	<0.01	<0.01	<0.01	<0.01
		Control	4	<0.01	<0.01	<0.01	<0.01
23-25 Oct 2010	+211-213	Movento	5	<0.01	<0.01	<0.01	<0.01
		Control	3	<0.01	<0.01	<0.01	<0.01

1 = enol metabolite is presented as the parent equivalent. The parent equivalent calculation = enol measured concentration x 1.36
2 = stored pollen is also referred to as hive-collected pollen or as pollen collected from the comb in this report; a.k.a bee bread.

B. Observations

No quantifiable residues of Movento were present in samples of stored pollen and honey collected from the study hives during the remainder of the study, or in the citrus blossoms of the study trees collected the following year. Based on the low levels of residues, it appears there is a high degree of safety with the use of Spirotetramat SC 240 G during citrus bloom. There were no significant differences between the control and treatment groups of hives for any colony strength and health measurements, and brood cohort success, during the in-citrus phase of the study. Both groups of hives started experiencing high losses of colonies between the blueberry pollination period and the fall colony assessments. *Varroa* mite and *Nosema* counts were high in both groups of hives throughout most of the study period, and deformed-wing condition accounted for almost one-third of intra-hive mortality before leaving citrus. Hive monitoring and assessment results strongly suggest that the primary causes of the high colony losses by fall, in both groups of hives, were *Varroa destructor*, *Nosema* spp., deformed-wing virus, queen issues (i.e. direct losses, failed replacement, poor performance), and multiple and various other pathogens and factors possibly to a lesser degree.

CONCLUSION

Based on the results of this study, it appears that Spirotetramat SC 240 G has a high margin of safety to honey bees when it is applied to citrus during bloom. Residue levels in pollen and nectar were well below levels of possible toxicological concern for both adult bees and bee brood. No adverse effects on the colonies exposed to Movento were noted in the Movento-exposed colonies, thus confirming that risks are minimal for this use pattern of Movento.

([redacted] R. E. L. & [redacted] G. R., 2010)

Report:	**KIIIA1 10.4.5/07, [redacted] S.; 2011**
Title:	Assessment of side effects of Spirotetramat SC 100 on the honey bee (*Apis mellifera* L.) applied twice at 100 g a.s./ha to strawberries in the field in Spain in 2009 Date: 2011-02-04
Organisation:	[redacted], Germany Bayer CropScience AG, [redacted], Germany
Report No.:	S09-00072; M-401434-01-1
Publication:	unpublished
Dates of experimental work:	2009-07-09 to 2010-01-25
Guidelines:	OEPP/EPPO No. 170 (3) (2001)
Deviations:	None
GLP:	Yes (certified laboratory)

Executive summary

The aim of the study was to determine the potential effects of Spirotetramat SC 100 on the honey bee (*Apis mellifera* L.) in a field test.

Spirotetramat SC 100 was applied twice during bee-flight at a rate corresponding to 100 g a.s./ha with an interval of 14 days to full flowering strawberries under field conditions at two locations in Spain. Fields of untreated strawberries served as control. Mortality and foraging activity of the bees was checked over 4 (1st replicate) and 5 (2nd replicate) days prior to the first application and followed up between the applications, and for 21 days after the second application. The condition of the colonies and the bee brood development were checked once before the application, once between both applications and four times afterwards (up to 28 days after the second application).

The test item treatment did not result in an adverse effect on honey bees, as determined by mortality and flight intensity. Differences of bee behaviour between the control and the treatment group were not observed. The condition of the exposed honey bee colonies was not affected by the treatment.

Two applications of Spirotetramat SC 100 in an interval of 14 days at a rate corresponding to 100 g a.s./ha during bee flight in full flowering strawberries did not cause any adverse effects to exposed honey bee colonies under field conditions.

MATERIAL AND METHODS

A Materials

1. Test material	Spirotetramat SC 100
Description	suspension, white
Lot/batch No.	2009-002098
Analytical content a.s.	102.5 g/L (9.50 % w/w)
Stability of test compound	Test item is considered stable under test conditions. Expiry date: 2010-03-20, when stored +2 °C to + 30 °C
2. Vehicle	Vehicle: 1st application: 100 g a.s./ha (based on the analysed content of active substance) in 500 L water/ha; 2nd application: 100 g a.s./ha (based on the analysed content of active substance) in 500 L water/ha
3. Test animals	
Species	Honey bee (*Apis mellifera* L.)
Age	colonies including immature and adult stages
Source	Honey bee colonies, normally developed, healthy and queen-right

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Collection	--
Environmental conditions	Two test fields in Picassent (replicate 1: test item: 3961 m²; control: 4823 m², Distance: 5 km) and in Quatretonda (replicate 2: test item: 3482 m²; control: 3198 m², Distance: 3.2 km), Valencia, Spain; test fields were isolated and not close to other flowering crops
Temperature	Replicate 1: 23.1 – 27.9°C (1st application) 25.6 – 28.9°C (2nd application) Replicate 2: 28.7 – 30.1°C (1st application) 24.8 – 28.4°C (2nd application)
Relative humidity	Replicate 1: 69 – 74% (1st application) 52 – 68% (2nd application) Replicate 2: 55 – 56% (1st application) 61 – 67% (2nd application)
Photoperiod	Length of the days
Ventilation	--

B Study design and methods

1. Experimental phase: July 9, 2009 - January 26, 2010

2. Experimental treatments

Test fields were two spatially separated replicates which were carried out in full flowering strawberries (*Fragaria ananassa*) in Spain. The first replicate was conducted near Picassent, comprised one test item treated field (3961 m²) and one corresponding untreated control field (4823 m²), both fields separated from one another by a distance of 5.0 km between. The second replicate close to the town Quatretonda also comprised one test item treated field (3482 m²) and one corresponding untreated control field (3198 m²), both fields separated from one another by a distance of 3.2 km. The Picassent and the Quatretonda test location were separated from one another by a distance of approx. 60 km. Six commercial bee colonies were placed at each field before the applications in the test item fields at full flowering of the crop. To ensure that the bees are exposed to the treatment in the test fields, detailed assessments of foraging activity were done before as well as after the application.

Control: Fields of untreated strawberries.

Test item: Spirotetramat SC 100 with an application rate of 100 g a.s./ha in 500 L water/ha.

Application of the test item in the field test: Spirotetramat SC 100 was applied to the crop twice during bee-flight at full flowering of the strawberries in an interval of 14 days using a knapsack sprayer.

3. Observations

The number of dead bees, flight intensity and the foraging activity of the bees were checked over 4 (1st replicate) and 5 (2nd replicate) days prior to the first application in the test fields and followed up over 14 days between both applications and for 21 days after the second application. The condition of the colonies and the bee brood development were checked once before the application, once between both applications and four times afterwards (up to 28 days after the second application). The behaviour of the bees on the crop and around the hve was observed on the days of exposure in the fields. For determination of residues of the test item strawberry blossoms, pollen and nectar were taken at three and two dates during the exposure in the fields.

Potential effects of the test item on the honey bees was evaluated by comparing the results of the test item treatment to those of the control treatment and by comparing the post-application results with the pre-application data.

No statistical analysis of the data was made since the study was not conducted in a replicated design (individual hives set up at the same location are not considered true replicates).

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RESULTS AND DISCUSSION

A. Findings

Honey bee mortality:
For toxicity to honey bees in the field test see the table below.

Toxicity to Honey Bees, Field Test

Test item		Spirotetramat SC 100			
Test object		*Apis mellifera*			
Exposure		Two applications of Spirotetramat SC 100 at full flowering of the crop during bee flight			
Replicate		**Replicate 1 S09-00072-01 (Picassent)**		**Replicate 2 S09-00072-02 (Quatretonda)**	
Treatment group		**Test item treatment**	**Control**	**Test item treatment**	**Control**
Application dates:		29 Jul 2009 12 Aug 2009	-	14 Jul 2009 28 Jul 2009	-
Application rate g a.s./ha [1)]		100	-	100	-
Spray volume per ha [L water/ha]		500	-	500	-
Mean mortality [dead bees/ colony/ assessment day]	Pre-appl. [DAA2 -18/-19 to -14ba]:	2.5	0.9	4.3	2.3
	Pre-appl. [DAA2 -14ba]:	1.0	0.2	1.7	0.8
	Post-appl. [DAA2 -14aa]:	0.7	1.2	1.8	1.2
	Pre-appl. [DAA2 -14aa to 0ba]:	0.8	0.8	1.0	0.6
	Pre-appl. [DAA2 0ba]:	0.8	0.5	0.3	0.2
	Post-appl. [DAA2 0aa]:	1.5	1.3	0.3	0.0
	Post-appl. [DAA2 0aa to +21]:	0.6	0.3	0.6	0.4
Mean flight intensity [forager bees/m^2]	Pre-appl. [DAA2 -18/-19 to -14]:	0.2	0.5	0.7	1.5
	Pre-appl. [DAA2 -14ba]:	0.0	0.0	0.2	1.6
	Post-appl. [DAA2 -14aa]:	0.3	0.3	2.4	1.1
	Pre-appl. [DAA2 -14aa to -0ba]:	0.4	0.7	4.1	2.4
	Pre-appl. [DAA2 0ba]:	0.4	0.2	0.8	0.2
	Post-appl. [DAA2 0aa]:	1.5	6.6	4.7	5.6
	Post-appl. [DAA2 0aa to +21]:	0.8	1.4	6.5	8.6

aa/ ba= after/before application
DAA2 = days after second application
1) referring to the analysed content of active substance

Replicate 1:
During the pre-application period the mean daily mortality was 0.9 dead adult bees/colony in the control group (C) and 2.5 dead adult bees/colony in the test item treated group (T). At the assessment on the day of the first application (DAA2 -14aa), the mean mortality in the test item treatment group was 0.7 dead adult bees/colony and in the control 1.2 dead adult bees/colony. Between both applications (DAA2 -14 to 0ba), the mean daily mortality was 0.8 dead adult bees/colony in both treatment groups. On the day of the second application (DAA2 0aa), the mean mortality was 1.3 dead adult bees/colony in the control group and 1.5 dead adult bees/colony in the test item treated group. The daily mean post-application mortality (DAA2 0aa to +21) in the test item treatment group T was 0.6 dead adult bees/colony and 0.3 dead adult bees/colony in the control group.
The occurrence of dead larvae and pupae was nearly zero throughout the study in both treatment groups.
On the linen sheets within the crop area of the test fields, the level of mortality after the second application (mean number of dead bees per day) was 0.0 in both treatment groups after the second application.

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Mortality figures were, under consideration of the natural variability in this endpoint, within the same range in the control and in the treatment group throughout the study. An adverse treatment effect was not seen at any time.

Replicate 2:
During the pre-application period the mean daily mortality was 2.3 dead adult bees/colony in the control group (C) and 4.3 dead adult bees/colony in the test item treated group (T). At the assessment on the day of the first application (DAA2 -14aa) the mean mortality in the test item treatment group was 1.8 dead adult bees/colony and in the control 1.2 dead adult bees/colony. Between both applications (DAA2 -14 to 0ba), the mean daily mortality was 0.6 dead adult bees/colony in the control group and 1.0 dead adult bees/colony in the test item treatment. On the day of the second application (DAA2 0aa), the mean mortality was 0.0 dead adult bees/colony in the control group and 0.3 dead adult bees/colony in the test item treated group. The daily mean post-application mortality (DAA2 0aa to +21) in the test item treatment group T was 0.6 dead adult bees/colony and 0.4 dead adult bees/colony in the control group.
The occurrence of dead larvae and pupae was nearly zero throughout the study in both treatment groups.
On the linen sheets within the crop area of the test fields, the level of mortality after the applications (mean number of dead bees per day) was 0.0 in both treatment groups after the second application.
Mortality figures were, under consideration of the natural variability in this endpoint, within the same range in the control and in the treatment group throughout the study. An adverse treatment effect was not seen at any time.

<u>Honey bee flight intensity:</u>
Replicate 1:
The daily mean flight intensity (forager bees/100m row/10 min) during the pre-application period, before the first application, was 0.2 in the test item treatment group T and 0.5 in the control group. The mean flight intensity after the first application on DAA2 -14aa was 0.3 forager bees/100 m row/10 min in the test item treatment field in comparison to 0.3 in the control group C. The daily mean flight intensity between the two applications (DAA2 -14 to 0ba) was 0.4 forager bees/100 m row/10 min in the test item treatment and 0.7 in the control. At the assessment after the second application (DAA2 0aa) was 1.5 forager bees/100 m row/10 min in the test item treatment group in comparison to 6.6 in the control group C. The daily mean post-application flight intensity (DAA2 0aa to +21) in the test item treatment group T was 0.8 forager bees/100 m row/10 min and 1.4 in the control group.
Foraging activity figures were, under consideration of the natural variability in this endpoint, within the same range in control and treatment group throughout the study. An adverse treatment effect was not seen at any time.

Replicate 2:
The daily mean flight intensity (forager bees/100 m row/10 min) during the pre-application period, before the first application, was 0.7 in the test item treatment group T and 1.5 in the control group. The mean flight intensity after the first application on DAA2 -14 was 2.4 forager bees/100 m row/10 min in the test item treatment field in comparison to 1.1 in the control group C. The daily mean flight intensity between the two applications (DAA2 -14 to 0ba) was 4.1 forager bees/100 m row/10 min in the test item treatment and 2.4 in the control. At the assessment after the second application (DAA2 0aa) was 4.7 forager bees/100 m row/10 min in the test item treatment field in comparison to 5.6 in the control group C. The daily mean post-application flight intensity (DAA2 0aa to +21) in the test item treatment group T was 6.5 forager bees/100 m row/10 min and 8.6 in the control group.
Foraging activity figures were, under consideration of the natural variability in this endpoint, within the same range in control and treatment group throughout the study. An adverse treatment effect was not seen at any time.

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Condition of the colonies and brood development:

Replicate 1:

The mean strength of the colonies (mean number of bees per colony) in the test item treatment group and in the control group was 10511 and 10374 bees per hive at the brood assessment before start of exposure. On the last assessment, 28 days after the second application, the mean strength of the colonies was 7110 bees per hive in the test item group and 7462 bees per hive in the control group.

On the first brood assessment, before start of exposure, the brood nest size (number of brood combs per colony) was 4.5 combs with brood in both treatment groups. At the last assessment on DAA2 +28, the brood nest size was 4.8 combs with brood in the test item treatment and 4.0 in the control.

All brood stages in all colonies of the treatment and control groups were present at the different assessment dates during the experimental phase of the study which shows that the colonies and the queens were in good condition during the observation period (except three hives in the control group and one hive in the test item treatment group which lost their queens). Before start of exposure, the mean percentage of comb area with egg, larval and pupal cells per hive was 4.0, 3.9 and 12.2 % in the test item treatment group hives, and 2.2, 3.7 and 11.5 % in the control hives, respectively. At the last assessment on DAA2 +28 the mean percentage of egg, larval and pupal cells per hive was 2.9, 3.3 and 9.1 % in the test item treatment group hives and 2.1, 2.4 and 9.2 % in the control hives, with no differences between either treatment or control groups, except the ones caused by loss of queen.

Before start of exposure, 20.1, 46.5 and 33.4 % of the comb area per hive was covered by brood, food and empty cells, respectively, in the test item treatment group hives, and 17.4, 51.7 and 30.9 % in the control hives. At the end of the study, 15.3, 51.3 and 33.4 % of the hive area was covered by brood, food and empty cells, respectively, in the test item treated hives, and 13.6, 47.1 and 39.3 % in the control hives.

The mean hive weight remained on the same level during the exposure in the strawberry fields with 33.6 kg/hive at start of exposure and 34.7 kg/hive at end of exposure in the test item treatment and with 35.4 kg/hive and 34.7 kg/hive in the control.

Replicate 2:

The mean strength of the colonies (mean number of bees) in the test item treatment group and in the control group was 9134 and 8861 bees per hive at the brood assessment before start of exposure. On the last assessment, 28 days after the second application, the mean strength of the colonies was 9203 bees per hive in the test item group and 7667 bees per hive in the control group.

On the first brood assessment, before start of exposure, the brood nest size (number of brood combs per colony) was 4.0 combs with brood in the test item group and 4.2 combs with brood in the control group. At the last assessment on DAA2 +28, the brood nest size was 4.2 combs with brood in the test item treatment and 3.7 in the control.

All brood stages in all colonies of the treatment and control groups were present at the different assessment dates during the experimental phase of the study which shows that the colonies and the queens were in good condition during the observation period. Before start of exposure, the mean percentage of comb area with egg, larval and pupal cells per hive was 3.0, 2.8 and 10.1 % in the test item treatment group hives, and 2.8, 2.4 and 11.0 % in the control hives, respectively. At the last assessment on DAA2 +28 the mean percentage of egg, larval and pupal cells per hive was 2.5, 1.5 and 8.1 % in the test item treatment group hives and 2.4, 2.7 and 8.5 % in the control hives with no differences between either treatment or control groups.

Before start of exposure, 15.8, 40.4 and 43.8 % of the comb area per hive was covered by brood, food and empty cells, respectively, in the test item treatment group hives, and 16.2, 43.3 and 40.5 % in the control hives. At the end of the study, 12.2, 28.1 and 59.7 % of the hive area was covered by brood, food and empty cells, respectively, in the test item treated hives, and 13.5, 32.0 and 54.5 % in the control hives.

The mean hive weight decreased during the exposure in the strawberry fields from 30.8 kg/hive at start of exposure to 27.3 kg/hive at end of exposure in the test item treatment and from 31.8 kg/hive to 27.6 kg/hive in the control.

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Honey bee behaviour in front of the colonies and within the crop:

All bees showed normal behaviour throughout the study in both replicates and in both treatment groups. No differences regarding the behaviour of the bees were observed between the test item treatment group T and the control group C.

Residue analysis:

Residues of spirotetramat (BYI 08330) and its metabolites BYI08330-enol, BYI08330-mono-hydroxy, BYI08330-ketohydroxy and BYI08330-enol-glucoside in/on strawberry flowers, nectar and pollen were quantified by reversed phase High Performance Liquid Chromatography coupled with electrospray and MS/MS-detection (HPLC-MS/MS), using stable labelled standard solutions as internal standard.

After measuring the residues of the single target analytes (i.e. spirotetramat and its metabolites), also the total residue of spirotetramat was calculated. The total residue of spirotetramat is defined as the sum of parent spirotetramat (BYI 08330) + BYI08330-enol + BYI08330-mono-hydroxy + BYI08330-ketohydroxy + BYI08330-enol-glucoside, expressed in equivalents of parent spirotetramat (BYI 08330). The limit of quantitation (LOQ) for the total residue of spirotetramat was 0.053 mg/kg.

In treated flower samples, the total residue of spirotetramat (BYI 08330) ranged from 0.082 mg/kg to 0.952 mg/kg. The total residue of spirotetramat (BYI 08330) in pollen and nectar, collected from the honey bee colonies exposed to the spirotetramat treated fields, was always < 0.053 mg/kg, respectively.

The total residue of spirotetramat (BYI 08330) in control samples, i.e. control flowers as well as nectar and pollen collected from honey bee colonies exposed to the control fields, was always < 0.053 mg/kg, respectively.

B. Observations

The test item treatment did not result in an adverse effect on honey bees, as determined by mortality and flight intensity. Differences of bee behaviour between the control and the treatment group were not observed. The condition of the exposed honey bee colonies as assessed by colony status, development of the brood as well as strength and size of the brood nest, was not affected by the treatment. No evidence of disturbance or termination of the brood development caused by the exposure to the treated crop was seen in the colonies of the test item group and the control colonies, respectively.

CONCLUSION

Two applications of Spirotetramat SC 100 in an interval of two weeks at a rate corresponding to 100 g a.s./ha during bee flight in full flowering strawberries did not cause any adverse effects to exposed honey bee colonies under field conditions.

([redacted] S., 2011)

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IIIA1 10.4.6 Investigation of special effects

Due to the findings presented above, no further studies are required. The Q_{HO} and Q_{HC} values are < 50.

Report:	**KIIIA1 10.4.6/01, [redacted] J., [redacted] H.J., [redacted] Ch. & [redacted] R.; 2008b**
Title:	Determination of Effects of Spirotetramat in Spiked Pollen to Honeybee Brood under Semi-Field Conditions (Trial 2008). Date: 2008-09-05
Organisation:	Bayer CropScience AG, [redacted], Germany
Report No.:	[redacted]/AM046; M-306791-01-1
Publication:	unpublished
Dates of experimental work:	May 28, 2008 to July 02, 2008 (biological assessments).
Guidelines:	special design, no standard guideline available
Deviations:	not applicable
GLP	no

Material and methods:

Pollen with the target concentrations of spirotetramat (parent) and spirotetramat-Enol (relation parent to metabolite 1:2) as given below was prepared. This approximate ratio has been observed in pollen samples of treated crops (see Table IIIA1 10.4.1-2 and respective studies)

Target Concentrations of Spirotetramat (Parent) and Spirotetramat-Enol in Spiked Pollen, respectively

Total Spirotetramat (parent + Enol) target concentration* [mg total a.s./kg]	Spirotetramat parent target concentration* [mg/kg]	Spirotetramat-Enol target concentration* [mg/kg]
untreated control	**-**	**-**
20	**6.67**	**13.33**
10	**3.33**	**6.67**
2	**0.67**	**1.33**

* nominal concentrations

The pollen spiking procedure was conducted 3 times in June 2008 in 3 subsequent weeks. Thereby, 3 subsequently spiked pollen batches were created to ensure that the test substance concentration in the pollen provided to the bees was as close as possible to the maximum target concentration in spite of possible degradation. The analytical verification of the target concentrations is presented in the analytical findings (see below).

For preparation of the highest concentration of 20 mg a.s./kg pollen, 300 g of homogenous untreated pollen was spiked with a BYI 08330 solution containing 2 parts BYI 08330-enol and 1 part BYI 08330 parent (2.07 BYI 08330 parent + 4.02 mg BYI 08330-enol dissolved in water). For spiking, the pollen was filled into an "Aeromat" operated with air pressure. While swirling the pollen, the BYI 08330 solution was sprayed onto the pollen. After application of the solution to the pollen, the pollen was dried by swirling in the "Aeromat". The spiked pollen containing 10 mg a.s./kg was prepared as described above for the highest concentration. In this case 1.035 mg BYI 08330 parent were mixed with 2.01 mg BYI 08330-enol and dissolved in water. The lower concentrations of 2 mg a.s./kg pollen was prepared by mixing spiked pollen of the next higher concentration with appropriate amounts of untreated pollen in the "Aeromat".

Small honeybee colonies (approx. 1,400 - 1,500 honeybees) were confined in 30 m² tunnels on a mulched winter wheat field near [redacted] (Germany). During the acclimatisation period of 7 days, the colonies

were fed with untreated pollen and untreated honey. During the treatment period the colonies were fed with spiked pollen at the concentrations given above and with untreated honey for 23 days. After end of confinement the bees were transferred to an open area where they were free to forage. For each test concentration with spiked pollen, 3 replicate tunnels were set up. Pollen was provided in a Petri-dish at the bottom inside the bee hive, and honey was provided in a Petri-dish placed inside the tunnel. During the study, the treated pollen was exchanged in each treatment group with fresh spiked pollen of the corresponding concentration in 2 to 3 day intervals. Three tunnels with untreated pollen served as controls.

The small bee colonies were examined for treatment-related effects over a period of 30 days after start of exposure. The main focus of the study was on brood development (eggs, larvae and pupae) of the colonies. Further endpoints assessed were foraging activity at the honey feeder set up inside the tunnel, as well as the consumption of honey from the feeder and the consumption of pollen from the pollen feeder placed inside the bee hive. Behavioural anomalies and mortality as well as size of pollen and honey stores in the hives and the hive weight development were assessed.

Observations:
The comb areas containing brood of all stages (eggs, larvae and pupae) fluctuated in all control and treated pollen treatment groups on the different assessment days. The fluctuations observed are well within the range of natural variability of these endpoints, and no treatment-related effect was seen in these endpoints.
No effect on brood and brood development was found after consumption of different concentrations of Spirotetramat + Spirotetramat-Enol in pollen.
No effect on the pollen and nectar storage behaviour of the honeybees was found in any of the treatment groups.
Comb cell production, foraging and flight activity and hive weight development were unaffected in the controls and all treatment groups.
No treatment-related effect on mortality of adults or pupae was found.
Pollen from the hive feeder was accepted for consumption in all treatment groups and it was found that the pollen and honey consumption were not influenced by the treatment.

Biological Findings:

Average of the 3 replicates (=3 hives) per treatment group					
Study day	**Parameter**	**Control**	**20 mg a.s./kg***	**10 mg a.s./kg***	**2 mg a.s./kg***
		Average size [%] of comb area per hive			
0 daa	**Egg deposition**	45.0	34.2	41.2	46.7
2 daa		40.8	23.8	32.3	45.0
7 daa		36.0	27.9	32.3	33.1
14 daa		25.9	15.0	20.0	20.4
21 daa		17.7	24.4	20.0	17.3
28 daa		22.5	17.7	18.8	14.2
0 daa	**Larval abundance**	14.4	17.3	14.2	11.1
2 daa		7.3	8.3	7.9	6.9
7 daa		3.2	3.1	3.1	2.1
14 daa		1.9	3.3	2.7	1.9
21 daa		1.9	2.1	2.5	1.7
28 daa		12.7	13.1	8.8	12.5
0 daa	**Pupal abundance**	0.0	0.0	0.0	0.0
2 daa		0.4	0.8	0.6	0.0
7 daa		5.6	7.3	6.7	4.0
14 daa		7.3	5.4	8.1	3.8
21 daa		1.5	2.7	2.9	1.5
28 daa		4.6	5.6	6.5	4.2

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Average of the 3 replicates (=3 hives) per treatment group					
Study day	**Parameter**	**Control**	**20 mg a.s./kg***	**10 mg a.s./kg***	**2 mg a.s./kg***
0 daa	**Total brood**	59.4	51.5	55.4	57.7
2 daa		48.6	32.9	40.9	51.9
7 daa		44.8	38.4	42.1	39.2
14 daa		35.1	23.8	30.8	26.1
21 daa		21.1	29.2	25.5	20.5
28 daa		39.8	36.4	34.0	30.9
0 daa	**Adult honeybees**	52.3	60.6	45.6	61.3
2 daa		56.9	59.2	52.3	59.6
7 daa		42.3	55.2	39.8	47.5
14 daa		40.4	46.2	38.8	46.2
21 daa		35.0	29.6	29.8	31.1
28 daa		20.2	15.7	16.1	16.2
0 daa	**Pollen stores**	0	0	0	0
2 daa		0	0	0	0
7 daa		0	0	0	0
14 daa		0	0	0	0
21 daa		0	0	0	0
28 daa		14.8	9.8	13.1	8.9
0 daa	**Honey stores**	8.3	11.5	4.2	1.0
2 daa		6.7	18.8	3.3	4.2
7 daa		18.8	26.5	13.3	13.5
14 daa		17.1	19.8	15.6	17.7
21 daa		34.4	36.7	30.4	33.8
28 daa		23.1	24.6	22.0	26.7
		Average size per comb [cm²]			
0 daa	**Size of comb area**	51.2	45.8	51.1	47.0
2 daa		52.5	47.6	48.3	43.5
7 daa		54.4	50.7	50.0	47.0
14 daa		54.6	53.5	52.1	48.5
21 daa		56.9	54.7	55.1	50.6
28 daa		67.9	63.0	63.8	59.2
		Average of the total number of adult bees per colony [n]			
pre-exposure	**Dead bees in front of hive**	3.7	6.3	2.7	5.7
exposure		4.3	5.3	5.3	7.0
pre-exposure	**Dead bees at tunnel edges**	33.7	38.7	34.4	41.3
exposure		106.0	112.3	105.0	102.0
pre-exposure	**Dead pupae in front of hive**	0.0	0.0	0.0	0.0
exposure		0.3	0.3	0.7	0.0
pre-exposure	**Average foraging activity at honey feeder**	10.2	15.2	9.8	11.7
exposure		44.0	49.7	45.7	48.1
		Average food consumption per colony [g]			
pre-exposure	**Pollen (total)**	6.0	2.7	7.1	2.2
exposure		14.3	13.3	16.8	7.3
pre-exposure	**Honey (total)**	32.8	54.6	26.6	28.2
exposure		296.3	320.1	309.1	307.6
		Average weight increase per hive [% of initial weight]			
-7 daa - 28 daa	**Hive weight increase**	+ 1.4	-2.0	+ 0.3	- 0.1

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daa: days after start of exposure *: nominal
pre-exposure: -6 to 0 daa (mortality) -6 to -1 daa (foraging) -7 to 0 daa (pollen/honey consumption)
exposure: 1 to 28 daa (mortality) 0 to 20 daa (foraging) -7 to 22/21 daa (pollen/honey consumption)

Analytical Findings:

Two pollen samples were taken and analysed to determine the achieved concentrations of BYI 08330 + BYI 08330-enol in the spiked pollen samples from each test concentration of each prepared batch. One sample was taken directly after the spiking process for each test concentration. The second samples from the first pollen batch were taken, when it was exchanged against the second pollen batch.

Analyzed concentrations of BYI 08330 + BYI 08330-enol in pollen [mg a.s. + enol/kg pollen]				
	Control	20 mg a.s./kg	10 mg a.s./kg	2 mg a.s./kg
Batch 1 (directly after spiking process)	< LOQ	7.655	12.157	2.029
Batch 1 (at batch exchange)	< LOQ	9.017	10.935	2.263
Batch 2 (directly after spiking process)	< LOQ	15.137	8.985	1.890
Batch 2 at batch exchange)	< LOQ	14.844	9.374	1.254
Batch 3 (directly after spiking process)	< LOQ	19.580	9.167	1.902
Batch 3 at batch exchange)	< LOQ	18.019	9.828	1.996

LOQ BYI 08330 = 0.01 mg/kg LOQ BYI 08330-enol = 0.01 mg/kg
LOD BYI 08330 = 0.001 mg/kg LOD BYI 08330-enol = 0.001 mg/kg

Remark: One of the three prepared batches of 20 mg a.s./kg had a lower percent recovery versus the nominal concentration that the other two batches. Since only one of the three batches was affected, and it was fed to the bees only between 0 daa and 7 daa, it is not considered to have a major impact on the overall conclusions of the study.

Conclusion: No effect on brood and brood development was found after feeding small honeybee colonies for 21 days with pollen spiked with a nominal concentration of 20 mg spirotetramat + spirotetramat-Enol/kg pollen. There were no adverse effects on comb development, hive weight development, honey and pollen storage behaviour and foraging activity. No effect on adult or pre-imaginal mortality was found.

([redacted] J. et al., 2008b)

Report:	**KIIIA1 10.4.6/02, [redacted] J., [redacted] Ch., [redacted] H.J., [redacted] Ch. & [redacted] R.; 2007**
Title:	Determination of Effects of BYI 08330 in Spiked Pollen to Honeybee Brood under Semi-Field Conditions (Trial 2006). Date: 2007-09-14
Organisation:	Bayer CropScience AG, [redacted], Germany
Report No.:	IA06DVG060G002; M-292891-01-1
Publication:	unpublished
Dates of experimental work:	June 17, 2006 - July 27, 2006
Guidelines:	No standard guideline available, test was especially designed for the purpose of this study

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Deviations: not applicable
GLP no

Material and methods:
Pollen with the target concentrations of BYI 08330 (parent) and BYI 08330-enol (relation parent to metabolite 2:1) as given below was prepared.

Target Concentrations of BYI 08330 (Parent) and BYI 08330-enol in Spiked Pollen

Total BYI 08330 (parent + enol) target concentration [mg total a.s./kg pollen]	BYI 08330 parent target concentration [mg/kg pollen]	BYI 08330-enol target concentration [mg/kg pollen]
untreated control	-	-
10	6.67	3.33
2	1.33	0.67
0.5	0.33	0.17
0.2	0.13	0.07
0.05	0.033	0.017

The pollen spiking procedure was conducted two times in two subsequent weeks (on 2006-06-26 and 2006-07-03). Thereby, two subsequent spiked pollen batches were created to ensure that the test substance concentration in the pollen was as close as possible to the maximum target concentration in spite of possible degradation. The verification of the target concentrations is presented in the analytical findings.

For preparation of the highest concentration of 10 mg a.s./kg pollen, 400 g of homogenous untreated pollen was spiked with a BYI 08330 solution containing 2 parts BYI 08330-enol and 1 part BYI 08330 parent (400 mg a.s./L) in water. For spiking, the pollen was filled into an "Aeromat" operated with air pressure. While swirling the pollen, the BYI 08330 solution was sprayed onto the pollen. After application of the solution to the pollen, the pollen was dried by swirling in the "Aeromat". The lower concentrations of 2, 0.5, 0.2 and 0.05 mg a.s./kg pollen were prepared by mixing spiked pollen of the next higher concentration with appropriate amounts of untreated pollen in the "Aeromat".

Small honeybee colonies (approx. 1,400-1,500 honeybees) were confined in 30 m² tunnels on a mulched rye field near [redacted] (Germany). After an acclimatisation period of 7 days (-7 daa - 0 daa), during which the colonies were fed with untreated pollen, the colonies were fed with pollen spiked with BYI 08330 + BYI 08330-enol at the concentrations described above for 22 days (0 daa - 21 daa). After end of confinement the bees were transferred to an open area where they were free to forage (21 daa - 28 daa). For each test concentration, one replicate tunnel was set up. One tunnel with untreated pollen served as control. Honey was provided as carbohydrate source *ad libitum*.

The small bee colonies were examined for treatment-related effects over a period of 28 days. The main focus of the study was on brood development (eggs, larvae and pupae) of the colonies. Further endpoints assessed were foraging activity at the pollen and honey feeders set up inside the tunnels, as well as consumption of honey and pollen at these feeders and the consumption of pollen from a feeder placed inside the bee hive. Behavioural anomalies and mortality as well as size of pollen and honey stores and the hive weight development were assessed.

Observations:

The comb area containing brood of all stages (eggs, larvae and pupae) was in largely the same range in all treatment groups on the different assessment days. Although the findings for larval abundance are different to interpret due to natural variability and lack of replication, a slight and transient treatment effect in the 10 mg a.s./kg treatment group between daa 3 and daa 14 cannot be excluded. Average abundance of honey bee larvae expressed as a % per comb side at the 10 mg a.s./kg treatment declined from 20.6 to 7.3 to 5.0 on daa 0, 3, and 7, respectively. However, by daa 14 larval abundance was 5.6 in comparison to a control value at daa 14 of 6.9. While a treatment-related effect could not be ruled out, natural variability may also be responsible for the effect. The unreplicated design of the study

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makes it difficult to determine the actual cause. In the other treatment groups, no consistent treatment effect was seen.

In all other brood-related endpoints, no effect on brood and brood development was found after consumption of different concentrations of BYI 08330 + BYI 08330-enol in pollen.

On the different assessment days, the size of the honey stores was in the same range for all treatment groups. Except for the assessment on 14 daa in the 0.5 mg a.s./kg treatment group, no pollen was stored until the end of the confinement of the bees in the tunnels. At study end, pollen stores were of a comparable size in all treatment groups. No effect on the storage behaviour of the honeybees was found.

Comb cell production, mortality, foraging activity and hive weight development were comparable between control and all treatment groups.

Pollen from the hive feeder was accepted for consumption in all treatment groups and it was found that the pollen and honey consumption were not influenced by the treatment.

Biological Findings:

Study day	Parameter	Control	10 mg a.s./kg	2 mg a.s./kg	0.5 mg a.s./kg	0.2 mg a.s./kg	0.05 mg a.s./kg
		[average % per comb side]					
0 daa	**Egg deposition**	25.0	15.6	21.9	28.1	26.3	28.8
28 daa		8.8	5	5.6	8.8	6.3	4.4
0 daa	**Larval abundance**	11.9	20.6	13.8	15.6	20.6	17.5
28 daa		23.8	23.8	24.4	26.3	23.1	25.0
0 daa	**Pupal abundance**	0.0	0.0	0.0	0.0	0.0	0.0
28 daa		24.4	20	25	26.3	21.9	25.6
0 daa	**Total brood**	36.9	36.2	35.7	43.7	46.9	46.3
28 daa		57.0	48.8	55.0	61.4	51.3	55.0
0 daa	**Pollen stores**	0.0	0.0	0.0	0.0	0.0	0.0
28 daa		11.3	10.6	13.8	7.5	14.4	15.0
0 daa	**Honey stores**	21.9	14.4	29.4	27.5	24.4	31.3
28 daa		25.6	35.0	28.8	30.0	27.5	27.5
		size [cm²]					
0 daa	**Size of comb area**	61.0	65.6	69.1	67.0	67.5	66.6
28 daa		93.6	93.0	94.5	96.0	94.5	96.0
		total number of bees [n]					
pre-exposure	**Dead bees in front of hive**	2	5	6	4	2	4
exposure		13	9	12	11	10	4
pre-exposure	**Dead bees at tunnel edges**	15	16	15	15	16	14
exposure		180	140	175	132	147	163
pre-exposure	**Foraging activity at pollen feeder [∑ of all observation periods]**	5	5	5	5	6	4
exposure		15	15	20	21	23	31
pre-exposure	**Foraging activity at honey feeder***	120	117	116	117	115	117
exposure		700*	700*	700*	700*	700*	700*
		[g]					
pre-exposure	**Pollen consumption at hive feeder [∑ of all observation periods]**	22	29.3	21.7	24.6	27.0	25.8
exposure		102	112.5	109.6	130.7	110.7	118.6
pre-exposure		101.6	99.1	93.7	120.8	106.6	110.6

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Study day	Parameter	Control	10 mg a.s./kg	2 mg a.s./kg	0.5 mg a.s./kg	0.2 mg a.s./kg	0.05 mg a.s./kg
exposure	**Honey consumption**	582	598.9	653.8	608.9	594.4	630.1
		[%]					
-7 daa - 28 daa	**Hive weight increase**	29.4	27.7	34.2	35.6	33.7	38.5

daa: days after start of exposure
pre-exposure period: -6 daa to 0 daa
exposure period: 0 daa to 21 daa
*: The value 700 is an estimation, based on the fact that the pollen feeder was completely occupied by honeybees during all of the assessments with approx. 50 individuals per assessment.

Analytical Findings:

Two pollen samples were taken and analysed to determine the achieved concentrations of BYI 08330 + BYI 08330-enol in the spiked pollen samples from each test concentration of each prepared batch. One sample was taken directly after the spiking process for each test concentration. The second samples from the first pollen batch were taken, when it was exchanged against the second pollen batch. The second sample of the second pollen batch was taken at the end of the exposure period.

Analyzed concentrations of BYI 08330 + BYI 08330-enol in pollen [mg a.s. + enol/kg pollen]						
	Control	10 mg a.s./kg	2 mg a.s./kg	0.5 mg a.s./kg	0.2 mg a.s./kg	0.05 mg a.s./kg
Batch 1 (directly after spiking process)	-	9.85	1.74	0.55	0.20	0.066
Batch 1 (before exchange with batch 2)	< LOQ	9.73	2.12	0.42	0.19	0.044
Batch 2 (directly after spiking process)	< LOQ	7.98	1.63	0.36	0.14	0.032
Batch 2 (at the end of exposure period)	< LOQ	7.30	1.63	0.40	0.15	0.037

LOQ BYI 08330 = 0.01 mg/kg   LOQ BYI 08330-enol = 0.01 mg/kg
LOD BYI 08330 = 0.001 mg/kg   LOD BYI 08330-enol = 0.001 mg/kg

Conclusion: A slight transient effect to larval abundance in the highest treatment group cannot be excluded, though data are not unequivocal. In the other treatment group, no consistent effects were seen in this endpoint. There were no adverse effects to other brood-related endpoints, comb development, hive weight development, honey and pollen storage behaviour, foraging activity and mortality by foraging on and consumption of pollen containing up to 10 mg BYI 08330 + BYI 08330-enol/kg pollen.

([redacted] J. et al., 2007)

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IIIA1 10.4.6.1 Larval toxicity

A brood feeding test according to Oomen et al. (1992) was conducted with the representative formulation Spirotetramat OD 100 ([redacted], 2004; KIIA 8.7.4/01; Document No.: M-000345-01-2) and also with the formulation Spirotetramat SC 240 ([redacted], 2004; KIIA 8.7.4/02; Document No.: M-121877-01-1).

IIIA1 10.4.6.2 Long residual effects

Due to the findings presented above, no further studies are required. The Q_{HO} and Q_{HC} values are < 50.

Report:	**KIIIA1 10.4.6.2/01, [redacted] H.J., [redacted] Ch. [redacted] R. & [redacted] J.; 2006a**
Title:	Residues of Spirotetramat and its metabolites in blossom samples of melons after spray application in Spain Not a Guideline Study (non-GLP) Date: 2006-03-15
Organisation:	Bayer CropScience AG; [redacted], Germany
Report No.:	IA05VSSSJAIN01; M-298511-01-1
Publication:	unpublished
Dates of experimental work:	Dates of biological work: 2005-08-05 to 2005-08-22 Dates of analytical work: 2005-08-22 (method validation) 2005-09-20 (last printout)
Guidelines:	Internal testing method, not a guideline study
Deviations:	not applicable
GLP	no

Material and methods:

Melons of the species *Cucumis melo* L. ssp. *melo*, variety "Cantalupo", were planted on a field at the "[redacted]" ([redacted], Spain). Six plots, each covering an area of 45 m², were separated on the melon field. The plants received a spray application with Spirotetramat OD 150, either at 72 g a.s./ha (treatment groups 4, 5 and 6) or at 288 g a.s./ha (treatment groups 1, 2 and 3). In treatment groups 1, 2, 4 and 5 all open blossoms were removed before the application to conduct a pre-flowering application, while in treatment groups 3 and 6 open blossoms were present for a full flowering application. For each application rate, 72 and 288 g a.s./ha, a sample of blossoms was taken at different times after the application (3 hours, 5 days and 10 days, respectively, after the application) to determine the residue levels of spirotetramat and its metabolites. Samples were stored at -20°C in an appropriate container. After the last sampling they were packed in a box with dry ice (temperature approx. -80°C) and were sent frozen via courier to BCS-RD-D-ROCS in 40789 [redacted] (Germany) for residue analysis.

The melon blossom samples were analysed for spirotetramat and its metabolites BYI 08330-enol, BYI 08330-keto-hydroxy, BYI 08330-mono-hydroxy and BYI 08330-enol-glucoside. Extraction, sample clean-up and determination of spirotetramat and its metabolites by HPLC-MS/MS were performed according to method 00857 (MR-099/04). The LOQ (limit of quantitation) was 0.010 mg/kg for spirotetramat and 0.012 mg/kg for the metabolites BYI 08330-enol, BYI 08330-keto-hydroxy and BYI 08330-mono-hydroxy. The LOQ for BYI 08330-enol-glucoside was 0.008 mg/kg.

Findings: In the following table the results of the residue analyses of the melon blossom samples are summarised.

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Analytical Findings in Melon Blossoms

Treatment group	Application rate [g a.s./ha]	Sampling time after application	Residue of spirotetramat and BYI 08330-enol [mg/kg]	Total Residue of spirotetramat* [mg/kg]
Control	-	not applicable	<LOQ	<LOQ
1	288	10 days	0.655	0.751
2	288	5 days	1.798	1.902
3	288	3 hours	4.643	4.690
4	72	10 days	0.170	0.192
5	72	5 days	0.423	0.442
6	72	3 hours	1.444	1.463

* Total residue = spirotetramat plus its metabolites BYI 08330-enol, BYI 08330-keto-hydroxy, BYI 08330-mono-hydroxy and BYI 08330-enol-glucoside
Total residues expressed as parent equivalent   LOQ = 0.0545 mg a.s./kg

([redacted] H.J., 2006a)

Report:	**KIIIA1 10.4.6.2/02, [redacted] H.J., [redacted] Ch. [redacted] R. & [redacted] J.; 2006b**
Title:	Residues of Spirotetramat and its metabolites in nectar and pollen samples of melons after spray application (tunnel test) in Spain Not a Guideline Study (non-GLP) Date: 2006-03-15
Organisation:	Bayer CropScience AG, [redacted], Germany
Report No.:	IA05VSSSJBJN01; M-298516-01-1
Publication:	unpublished
Dates of experimental work:	Dates of biological work: 2005-08-11 to 2005-08-29 Dates of analytical work: 2005-08-22 (method validation) 2005-09-20 (last printout)
Guidelines:	Internal testing method, not a guideline study
Deviations:	not applicable
GLP	no

Material and methods:

Melons of the species *Cucumis melo* L. ssp. *melo*, variety "Cantalupo", were planted on a field at the "[redacted]" ([redacted], Spain). Two tunnels, each covering an area of 300 m², were set up on the melon field.
The melon plants inside the tunnels received either a pre-flowering spray application with Spirotetramat OD 150 at 288 g a.s./ha (treatment group 1) or a spray application during full flowering at 96 g a.s./ha (treatment group 2).
Honey bees were used as sampling agents for nectar and pollen. Into each tunnel (one per treatment group) one small honey bee colony with approx. 3,000 bees and a queen of the sub-species *Apis mellifera iberica* was set up. The hives comprised of 5 frames, of which two were empty frames.
In each treatment group samples of nectar were taken from the cells surrounding the brood nest. Pollen samples were cut out from the new combs of the formerly empty frames using a scalpel. This was conducted on the days following hive introduction (DAI), respectively days after treatment (DAT):
For treatment group 1 the sampling was conducted on DAI 6 = DAT 11 and on DAI 12 = DAT 17.

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For treatment group 2 the sampling was conducted on DAI 6 = DAT 5 and on DAI 12 = DAT 11.
All samples were stored at -20°C in an appropriate container. After the last sampling they were packed in a box with dry ice (temperature approx. -80°C) and were sent frozen via courier to BCS-RD-D-ROCS in 40789 [redacted] (Germany) for the residue analysis.
The nectar and pollen samples were analysed for spirotetramat and its metabolites BYI 08330-enol, BYI 08330-keto-hydroxy, BYI 08330-mono-hydroxy and BYI 08330-enol-glucoside. Extraction, sample clean-up and determination of spirotetramat and its metabolites by HPLC-MS/MS were performed according to method 00857 (MR-099/04). The LOQ (limit of quantitation) was 0.010 mg/kg for spirotetramat and 0.012 mg/kg for the metabolites BYI 08330-enol, BYI 08330-keto-hydroxy and BYI 08330-mono-hydroxy. The LOQ for BYI 08330-enol-glucoside was 0.008 mg/kg.

Findings: In the following table the results of the residue analyses of the melon nectar and pollen samples are summarised.

Analytical Findings in Melon Nectar and Pollen

Sample type	Treatment group	Application rate [g a.s./ha]	Application time	Days after treatment (DAT)*	Days after hive introduction (DAI)	Residue of spirotetramat and BYI 08330-enol [mg/kg]	Total Residue of spirotetramat ** [mg/kg]
nectar	1	288	5 days before flowering	11	6	<LOQ	<LOQ
				17	12	<LOQ	<LOQ
	2	96	at full flowering	5	6	<LOQ	<LOQ
				11	12	<LOQ	<LOQ
pollen	1	288	5 days before flowering	11	6	0.016	0.030
				17	12	0.306	0.306
	2	96	at full flowering	5	6	0.180	0.180
				11	12	0.213	0.213

* First sampling of nectar and pollen for both treatment groups on day 2005-08-23.
** Total residue = spirotetramat plus its metabolites BYI 08330-enol, BYI 08330-keto-hydroxy, BYI 08330-mono-hydroxy and BYI 08330-enol-glucoside
Total residues expressed as parent equivalent LOQ = 0.0545 mg a.s./kg

([redacted] H.J., 2006b)

Report: **KIIIA1.10.4.6.2/03, [redacted] H.J., [redacted] Ch. [redacted] R. & [redacted] S.; 2007a**
Title: Residues of Spirotetramat and its metabolites in nectar and pollen of citrus after spray application in Spain
Not a Guideline Study (non-GLP)
Date: 2007-11-29
Organisation: Bayer CropScience AG, [redacted], Germany
Report No: IA07VSSH6SJN01; M-295273-01-1
Publication: unpublished
Dates of experimental work: Dates of biological work: 2007-03-19 to 2007-05-04
Dates of analytical work: 2007-06-13 to 2007-06-20
Guidelines: Internal testing method, not a guideline study
Deviations: not applicable

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GLP no

Material and methods:
Citrus trees of the species *Citrus*, spec., variety clemenules, were planted on 1998-04-02 on a field at the "[redacted]" ([redacted], Spain). At study initiation, the tree age was 9 years and the tree height 2.5 m (1.5 m canopy height). A tunnel covering an area of 337.5 m² was set up in the citrus orchard covering 27 trees.
The citrus trees inside the tunnel received two spray applications with Spirotetramat OD 150, one at 75 g a.s./ha/canopy height on 2007-03-19 (pre-blossom, first flower buds visible; BBCH 53), a second at 75 g a.s./ha/canopy height on 2007-04-27 (sufficient flowering to feed the bee colony, BBCH 65). This rate is equivalent to 112.5 g a.s./ha when adjusted for canopy height.
Honey bees were used as sampling agents for nectar and pollen. A honey bee colony (*Apis mellifera iberica*) with approx. 4,000 - 5,000 bees was set up in the tunnel one day after the last application. The hives comprised of 5 combs, of which two were empty combs. From these empty combs later samples of the freshly collected citrus nectar were taken.
The sampling of nectar was conducted 7 days after the second application (day 46; 2007-05-04) (Samples "honey 30 treated", "honey 31 treated" and "honey 35 treated"). Nectar was taken from the originally empty comb using a single-use syringe with an appropriately sized cannula.
No pollen could be collected from the honey combs, as originally intended, since citrus pollen was not collected and stored by the bees in sufficient quantities for sampling and residue analysis.
All samples were stored at -20°C in an appropriate container after sampling. After the last sampling they were packed in a box with dry ice (temperature approx. -80°C) and were sent frozen via courier to BCS-D-ROCS ([redacted], Germany) for residue analysis.
The nectar samples were analyzed for spirotetramat and its metabolites BYI 08330-enol, BYI 08330-keto-hydroxy, BYI 08330-mono-hydroxy and BYI 08330-enolglucoside.
Extraction, sample clean-up and determination of spirotetramat and its metabolites by HPLC-MS/MS were performed according to method 00857 (MR-099/04). The LOQ (limit of quantification) was 0.010 mg/kg for spirotetramat and 0.012 mg/kg for the metabolites BYI 08330-enol, BYI 08330-keto-hydroxy and BYI 08330-mono-hydroxy. The LOQ for BYI 08330-enol-glucoside was 0.008 mg/kg.

Findings: In the following table the results of the residue analyses of citrus nectar are summarized.

Summary - Analytical Findings in Citrus Nectar

Treatment group	Applications	Application rate [g a.s./ha/m canopy height]	Canopy height [m]	Sampling time after the second application [d]	Total Residue of spirotetramat, mean of all samples* [mg/kg]
1	A: pre-blossom (2007-03-19) B: full flowering (2007-04-27)	75 75	1.5	7	< LOQ

* Total residue = spirotetramat plus its metabolites BYI 08330-enol, BYI 08330-keto-hydroxy, BYI 08330-mono-hydroxy and BYI 08330-enol-glucoside
Total residues expressed as parent equivalent LOQ = 0.0545 mg a.s./kg

([redacted] H.J., 2007a)

Report: **KIIIA1 10.4.6.2/04, [redacted] H.J., [redacted] Ch. [redacted] R. & [redacted] S.; 2007b**
Title: Residues of Spirotetramat and its metabolites in citrus blossoms after spray application in Spain
Not a Guideline Study (non-GLP)
Date: 2007-11-29

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Organisation:	Bayer CropScience AG, [redacted], Germany
Report No.:	IA07VSSH7SJN01; M-295276-01-1
Publication:	unpublished
Dates of experimental work:	Dates of biological work: 2007-03-16 to 2007-05-04 Dates of analytical work: 2007-06-13 to 2007-06-20
Guidelines:	Internal testing method, not a guideline study
Deviations:	not applicable
GLP	no

Material and methods:
Citrus trees of the species *Citrus* spec., variety clemenules, were planted on 1998-04-02 on a field at the "[redacted]" ([redacted], Spain). At study initiation, the tree age was 9 years and the tree height 2.5 m (1.5 m canopy height).
One plot covering an area of 100 m² was separated on the citrus plantation (20 trees in a row). The trees received two spray applications with Spirotetramat OD 150, one at 75 g a.s./ha/canopy height on 2007-03-16 (pre-blossom, BBCH 53), a second at 75 g a.s./ha/canopy height on 2007-04-27 (full flowering, BBCH 65). This rate is equivalent to 112.5 g a.s./ha when adjusted for canopy height.
Open entire blossoms were sampled 7 days after the second application to determine the residue levels of spirotetramat and its metabolites. Samples were stored at -20°C in an appropriate container. After the last sampling they were packed into a box with dry ice (temperature approx. -80°C) and were sent frozen via courier to BCS-D-ROCS in [redacted] /Germany) for residue analysis.
The citrus blossom samples were analyzed for spirotetramat and its metabolites BYI 08330-enol, BYI 08330-keto-hydroxy, BYI 08330-mono-hydroxy and BYI 08330-enolglucoside.
Extraction, sample clean-up and determination of spirotetramat and its metabolites by HPLC-MS/MS were performed according to method 00857 (MR-099/04). The LOQ (limit of quantification) was 0.010 mg/kg for spirotetramat and 0.012 mg/kg for the metabolites BYI 08330-enol, BYI 08330-keto-hydroxy and BYI 08330-mono-hydroxy. The LOQ for BYI 08330-enol-glucoside was 0.008 mg/kg.

Findings: In the following table the results of the residue analyses of citrus blossom samples are summarized.

Summary - Analytical Findings in Citrus Blossoms

Treatment group	Applications	Application rate [g a.s./ha/m canopy height]	Canopy height [m]	Sampling time after the second application [d]	Residues of spirotetramat and BYI 08330-enol [mg/kg]	Total Residue of spirotetramat* [mg/kg]
1	A: pre-blossom (2007-03-16) B: full flowering (2007-04-27)	75 75	1.5	7	Mean ± SD: 0.506 ± 0.102 Minimum/ Maximum: 0.395/ 0.641	Mean ± SD: 0.591 ± 0.100 Minimum/ Maximum: 0.463/ 0.742

* Total residue = spirotetramat plus its metabolites BYI 08330-enol, BYI 08330-keto-hydroxy, BYI 08330-mono-hydroxy and BYI 08330-enol-glucoside
Total residues expressed as parent equivalent LOQ = 0.0545 mg a.s./kg

([redacted] H.J., 2007b)

Report:	**KIIIA1 10.4.6.2/05, [redacted] H.J., [redacted] Ch. [redacted] R. & [redacted] S.; 2007c**
Title:	Residues of Spirotetramat and its metabolites in nectar and pollen of

	phacelia after spray application in Germany Not a Guideline Study (non-GLP) Date: 2007-11-29
Organisation:	Bayer CropScience AG, [redacted], Germany
Report No.:	IA07DVG015G001; M-295137-01-1
Publication:	unpublished
Dates of experimental work:	Dates of biological work: 2007-07-12 to 2007-08-11 Dates of analytical work: 2007-11-07 to 2007-11-12
Guidelines:	Internal testing method, not a guideline study
Deviations:	not applicable
GLP	no

Material and methods:

Phacelia tanacetifolia BENTH. was sown on 2007-04-26 on a field at "[redacted]", [redacted], Germany. Crop emergence was observed on 2007-05-10. Two tunnel tents each covering an area of 100 m² (width x length: 5 m x 20 m) were set up in the test field. The phacelia plants inside the tunnels received one spray application with Spirotetramat OD 150 at 100 g a.s./ha on 2007-07-13 (full flowering, BBCH stage 65).

Honey bees were used as sampling agents for nectar and pollen. One honey bee colony of the species *Apis mellifera* with approx. 3,000-3,500 bees was set up in each tunnel at the day of the application (morning). At this point of time the entrances of the hives were closed. A hive comprised of 3 combs with a size of 35 cm x 20 cm.

After the application of the test item the bee hive entrances were opened and the middle comb was exchanged with an empty comb in each hive. From these empty combs later samples of the freshly collected phacelia nectar and pollen were taken.

The sampling of nectar and pollen was conducted 4, 6, 7 and 10 days after the application (2007-07-17, 2007-07-19, 2007-07-20 and 2007-07-23). Nectar and pollen were sampled using a single-use syringe with an appropriately sized cannula.

All samples were stored at -20°C in an appropriate container. After the last sampling they were packed in a box with dry ice {temperature approx. -80°C) and transported to BCS-D-ROCS ([redacted], Germany) for residue analysis.

The nectar and pollen samples were analyzed for spirotetramat and its metabolites BYI 08330-enol and BYI 08330-ketohydroxy. Extraction, sample clean-up and determination of spirotetramat and its metabolites by HPLC-MS/MS were performed according to method 00857 (MR-099/04). The LOQ (limit of quantitation) was 0.01 mg/kg for spirotetramat and its metabolites.

Additionally, mortality, hive weight, colony strength and brood development of the bee colonies were assessed during the study.

Findings:

In the following table the results of the residue analyses of pollen and nectar of phacelia are summarized.

Summary - Analytical Findings in Pollen and Nectar of Phacelia

Sample type	Application rate	Date of sampling	Days after application	Residues of spirotetramat and BYI 08330-enol Mean (minimum – maximum) [mg/kg]	Total Residue of spirotetramat* Mean (minimum – maximum) [mg/kg]
Pollen	100 g a.s./ha	2007-07-17	4	5.76 (5.03 - 6.12)	5.77 (5.18 - 6.35)
		2007-07-19	6	3.33 (2.12 - 4.54)	3.44 (2.19 - 4.69)
		2007-07-20	7	3.87 (3.73 - 4.00)	3.99 (3.85 - 4.12)
		2007-07-23	10	3.88 (3.46 - 4.30)	4.09 (3.66 - 4.51)
Nectar	100 g a.s./ha	2007-07-17	4	0.27 (0.23 - 0.31)	0.29 (0.24 - 0.33)

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		2007-07-19	6	0.27 (0.24 - 0.30)	0.29 (0.26 - 0.32)
		2007-07-20	7	0.23 (0.22 - 0.23)	0.24 (0.24 - 0.24)
		2007-07-23	10	0.21 (0.20 - 0.21)	0.22 (0.21 - 0.23)

* Total residue = spirotetramat plus its metabolites BYI 08330-enol, BYI 08330-keto-hydroxy, BYI 08330-mono-hydroxy and BYI 08330-enol-glucoside
Total residues expressed as parent equivalent LOQ = 0.0545 mg a.s./kg

The results show that total residues in pollen are distinctly higher than residues in nectar. In pollen, residue levels were highest on sampling day 4, and lower on the subsequent sampling days, but generally within the same order of magnitude on the different sampling days. In nectar, residue levels on the different sampling days were within the same order of magnitude. Furthermore, only the two metabolites of spirotetramat were detected in nectar of phacelia, whereas the parent and both metabolites were found in pollen. In all pollen samples, the enol metabolite made up the greatest proportion of the total residues; the parent compound and the ketohydroxy metabolite were detected in significantly smaller quantities. No obvious or significant adverse effects of 100 g spirotetramat/ha on mortality of adult bees and pupae, bee hive weight, colony strength and bee brood development were observed.

([redacted] H.J., 2007c)

Report:	**KIIIA1 10.4.6.2/06, [redacted] H.J., [redacted] Ch. [redacted] R. & [redacted] S., 2007d**
Title:	Residues of Spirotetramat and its metabolites in nectar and pollen of summer oilseed rape after spray application in Germany Not a Guideline Study (non-GLP) Date: 2007-11-29
Organisation:	Bayer CropScience AG, [redacted], Germany
Report No.:	IA07DVG015G002; M-295271-01-1
Publication:	unpublished
Dates of experimental work:	Dates of biological work: 2007-07-12 to 2007-08-11 Dates of analytical work: 2007-11-07 to 2007-11-12
Guidelines:	Internal testing method, not a guideline study
Deviations:	not applicable
GLP	no

Material and methods:
Summer oilseed rape of the species *Brassica napus* L. spp. napus (spring), variety "Ability", was sown on 2007-04-26 on a field at "[redacted], Germany.
Crop emergence was observed on 2007-05-10. Two tunnel tents each covering an area of 100 m² (width x length: 5 m x 20 m) were set up in the test field. The summer oilseed rape plants inside the tunnels received one spray application with Spirotetramat OD 150 at 100 g a.s./ha on 2007-07-13 (full flowering, BBCH stage 65).
Honey bees were used as sampling agents for nectar and pollen. One honey bee colony of the species Apis mellifera with 3,000-3,500 bees was set up in each tunnel at the day of the application (morning). At this point of time the entrances of the hives were closed. A hive comprised of 3 combs with a size of 35 cm x 20 cm. After the application of the test item the bee hive entrances were opened and the middle comb was exchanged with an empty comb in each hive. From these empty combs later samples of the freshly collected rape nectar and pollen were taken.
The sampling of nectar and pollen was conducted 4, 6 and 7 days after the application (2007-07-17, 2007-07-19 and 2007-07-20). Nectar and pollen were sampled using a single-use syringe with an appropriately sized cannula.

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All samples were stored at -20°C in an appropriate container. After the last sampling they were packed in a box with dry ice (temperature approx. -80°C) and transported to BCS-D-ROCS ([redacted], Germany) for residue analysis.
The nectar and pollen samples were analyzed for spirotetramat and its metabolites BYI 08330-enol and BYI 08330-ketohydroxy. Extraction, sample clean-up and determination of spirotetramat and its metabolites by HPLC-MS/MS were performed according to method 00857 (MR-099/04). The LOQ (limit of quantitation) was 0.01 mg/kg for spirotetramat and its metabolites.
Additionally, mortality, hive weight, colony strength and brood development of the bee colonies were assessed during the study.

Findings:
In the following table the results of the residue analyses of pollen and nectar of summer oilseed rape are summarized.

Summary - Analytical Findings in Summer Oilseed Rape Pollen and Nectar

Sample type	Application rate	Date of sampling	Days after application	Residues of spirotetramat and BYI-08330-enol Mean (minimum – maximum) [mg/kg]	Total Residue of spirotetramat* Mean (minimum – maximum) [mg/kg]
Pollen	100 g a.s./ha	2007-07-17	4	6.19 (5.47 – 6.90)	6.65 (5.94 – 7.36)
		2007-07-19	6	6.95 (6.23 – 7.67)	7.60 (6.89 – 8.31)
		2007-07-20	7	6.23 (6.10 – 6.36)	6.88 (6.61 – 7.14)
Nectar	100 g a.s./ha	2007-07-17	4	0.05 (0.04 – 0.06)	0.05 (0.04 – 0.06)
		2007-07-19	6	0.05 (0.04 – 0.06)	0.05 (0.04 – 0.06)
		2007-07-20	7	0.04 (0.04 – 0.04)	0.04 (0.04 – 0.04)

* Total residue = spirotetramat plus its metabolites BYI 08330-enol and BYI 08330-ketohydroxy
Total residue expressed as parent equivalent LOQ = 0.0545 mg a.s./kg

The results showed that total residues in pollen are distinctly higher than residues in nectar. In both matrices, residue levels on the different sampling days were within the same orders of magnitude. No parent compound but only the enol metabolite was detected in nectar of summer oilseed rape, whereas the parent compound and both metabolites were found in pollen. In all pollen samples, the enol metabolite made up the greatest proportion of the total residues; the parent compound and the ketohydroxy metabolite were detected in significantly smaller quantities.
No obvious adverse effects of 100 g spirotetramat/ha on mortality of adult bees and pupae, bee hive weight, colony strength and bee brood development were observed.

([redacted] H.J., 2007d)

IIIA1 10.4.6.3 Disorienting effects on bees

Covered by the field studies conducted. See point IIIA1 10.4.7 below.

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

Report:	**KIIIA1 10.4.7/01, [redacted]; 2005**
Title:	Assessment of side effects of BYI 08330 OD 100 on the honey bee (*Apis mellifera* L.) in the semi-field. Date: 2005-02-02
Organisation:	[redacted], Germany Bayer CropScience AG, [redacted], Germany
Report No.:	20041144/01-BZEU; M-244490-01-1
Publication:	unpublished
Dates of experimental work:	May 17, 2004 -July 06, 2004
Guidelines:	EPPO No. 170 (3) Guideline on test methods for evaluation the side-effects of plant protection products on honey bees (OEPP/EPPO, 2001) and the Bulletin of Insectology 56 (1), 91 – 96; 2003: Honey bee brood ring-test in 2002: method for the assessment of side effects of plant protection products on the honey bee brood under semi-field conditions.
Deviations:	no
GLP:	yes (certified laboratory)

Executive summary

The objective of the study was to determine the effects of BYI 08330 OD 100 on the honey bee, *Apis mellifera* under semi-field conditions in a tunnel test. Most attention was directed to the development of the bee brood. The semi-field study consisted of 4 treatment groups; the test item treatment group 1 where BYI 08330 OD 100 was applied five times, the test item treatment group 2 where BYI 08330 OD 100 was applied once, the water treated control and the toxic standard treatment (Insegar WG 25), Separate tunnels (3 replicates per treatment group) were set-up in a field of flowering oil-seed spring-rape *(Brassica napus)*. It was concluded that the application of BYI 08330 OD 100 applied five times (treatment group T1) and once (treatment group T2) on a bee-attractive flowering crop such as oil-seed spring-rape did not result in an adverse effect on the mortality of adult honey bees and brood, flight intensity of the bees on the crop and the behaviour of the bees in the crop area or in front of the hive.

MATERIAL AND METHODS

A Materials

1. Test material	BYI 08330 OD 100
Description	Liquid, brown suspension
Lot/batch No.	Batch no.: 08030/0110(0073) Tox no.: 06593-00
Content a.s.	102.02 g/L (analysed)
Stability of test compound	Sufficiently stable in spray solution (at least 1 hour)
2. Vehicle and/or positive control	Water Insegar WG 25 (a.s.: fenoxycarb, 25%)
3. Test animals	
Species	Honey bee (*Apis mellifera* L.), For the test, small healthy colonies ["Mini-Plus-Beuten"; 1 queen and approximately 1 kg bees (6000 - 8000 bees per colony)] with 12 combs were used.

B Study design and methods

1. In life dates	May 17, 2004 – July 06, 2004
2. Experimental treatments	

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The semi-field test was located in the south of Germany in the region of [redacted] near [redacted]. The crop used was oil-seed spring rape *(Brassica napus)* a crop specifically recommended in OEPP/EPPO Guideline No. 170 (3) for tunnel testing. Before full flowering and start of bee-exposure tunnel tents were set-up in the test field. The area covered with rape was 47.52 m^2 per tunnel. The tunnels had a size of 5.0 x 12 m and a height of about 3.5 m. The tunnel frames were covered with light plastic gauze (mesh size: 1.5 mm). Before the start of bee-exposure in the tunnels a path was created along the tunnel walls at both front sides and through the middle of each tunnel by removing the plants and levelling the ground. Subsequently, the path was covered with linen sheets. For the test, small healthy colonies ["Mini-Plus-Beuten"; 1 queen and approximately 1 kg bees (6000-8000 bees per colony)] with 12 combs were used. All nuclei were produced at the same time. The corresponding queens originated from one breeding line in order to guarantee uniform bee material in all treatment groups. Each bee hive consisted of two bodies with one bottom and one lid (height: 40 mm). The outer dimensions of this hive were 300 mm x 300 mm, the inner dimensions are 230 mm x 230 mm and the height is 170 mm. Each body contained six frames (130 mm x 200 mm). Wooden bee traps with gauze on bottom and on the top were attached to the entrance of the hives in order to register those dead bees which were carried out of the hives.

In test item treatment group T1, BYI 08330 OD 100 was applied three times on the crop before flowering at a rate of 72 g a.s. (692.33 g product)/ha in 400 L water/ha and furthermore two times after set-up of the colonies ("Mini-Plus-Beuten") in the tunnels on the flowering oil-seed spring-rape at a rate of 96 g a.s. (923.11 g product)/ha in 400 L water/ha. In treatment group T2 the test item was applied once, three days after set-up of the colonies in the tunnels at a rate of 96 g a.s./ha in 400 L water/ha. The control (water treated) and toxic standard (Insegar WG 25) applications were performed on the same day as the forth test item application in treatment group T1 (main application day). There were three tunnels (one hive per tunnel) per treatment.

3. Observations

Mortality, flight intensity, condition of the colonies and the development of the bee brood was assessed before and after the main application day of the test item. Particular attention was directed to the brood development of the colonies.

The influence of the test item was evaluated by comparing the results in the tunnel tents of the test item treatments to the control data and those of the toxic standard treatment. The following points were assessed:

- Condition of the colonies (strength) and development of the bee brood
- Mortality at the edge of the treated area and in the bee traps
- Foraging activity (number of forager bees/m^2 flowering rape)
- Behaviour of the bees on the crop and around the hive

Bee traps with gauze on the bottom and on the top were attached to the entrance of the hives in order to register those dead bees which were carried out of the hives. Furthermore, the mortality was recorded in the crop. Therefore the oilseed spring rape *(Brassica napus)* was removed prior to the set-up of the hives and water-permeable linen sheets (covered area approximately 15 m^2) were spread out in the array. The mortality of adult honey bees found on the linen sheets and in the bee trap at the entrance of the colonies was assessed according to the time schedule given below. After an exposure period of 10 days the bee colonies were taken out of the tunnels and transferred to an area without flowering main crops and where no pesticides were applied during the time of assessments. Mortality of adult honey bees in the bee trap at the entrance of the colonies was assessed for another 13 days [until day +23 after BFD (brood area fixing day)].

<u>Evaluation of mortality</u>

Time of the test　　　　　　　　　　　　Evaluations of mortality*

On the days after set-up of colonies in the tunnels up to day before main application day**	Once a day at the same time of day in the morning
Main application day **	• Shortly before application • 2 h after application • in the evening after daily bee flight
Day of 5th application in the test item treatment group T1	Once a day at the same time in the morning, before start of application
During the exposure days in the tunnels between and after applications until day 10 after BFD	Once a day at the same time of day in the morning
Up to day +23 after BFD (out of the tunnels; only be traps)	Once a day at the same time of day in the morning

* Remark: At each evaluation date the dead bees were counted and removed
BFD = Brood area fixing day
** Main application day = 09JUN04, the day of 4th application in the test item treatment group T1, and the application in the test item treatment group T2, the control and the toxic standard treatment

Evaluation of flight intensity

Time of the test	Evaluations of mortality*
On the days after set-up of colonies in the tunnels up to day before main application day**	Once a day during flight activity of the bees
Main application day **	• Shortly before application • four times in the first hour after application • 2 h after application • 4 h after application • 6 h after application
First day after main application day**	Three times during flight activity of the bees (Morning, midday, evening)
Day of 5th application in the test item treatment group T1	Once a day during flight activity of the bees, before start of application
During the exposure days in the tunnels between and after applications	Once a day during flight activity of the bees

** Main application day = 09JUN04, the day of 4th application in the test item treatment group T1, and the application in the test item treatment group T2, the control and the toxic standard treatment

Condition of the Colonies:
The condition of all colonies was checked two days before the main application day and five times afterwards (4, 9, 16, 23 and 29 days after BFD).

Assessment of the development of the bee brood:
Assessment of the development of the bee brood took place at the BFD (egg), 4 days ([redacted] to old larvae), 9 days (capped cells), 16 days (capped cells shortly before hatch) and 23 days after BFD (empty cells or cells containing eggs).

RESULTS AND DISCUSSION

A. Findings

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The test was considered to be valid because a clearly detectable effect of the toxic standard was found (e. g. brood termination before a successful hatch, significantly increased mortality of pupae).

Test substance		BYI 08330 OD 100			
Test object		*Apis mellifera*			
Exposure		T1: five spray treatments of BYI 08330 OD 100 three before and two during foraging activity in flowering oilseed spring rape T2: one spray treatment of BYI 08330 OD 100 during foraging activity in flowering oil-seed spring rape			
Treatment group		Test substance (BYI 08330 OD 100)		Control (water)	Toxic standard (Insegar 25 WG)
		T1	T2	C	R
Single application rate [in 400 L water/ha]		3 × 72 g a.s./ha 2 × 96 g a.s./ha	96 g a.s./ha	-	150 g a.s.
Mean Mortality [dead bees/ replicate/day]	pre	13.8	12.9	7.9	11.2
	post (BFD +2aa)	5.0	2.7	2.7	1.7
	post (BFD +2aa to +23)	5.5	4.5	4.1	8.1
	$Q_{M(average)}$:	0.4	0.3	0.5	0.7
∑ dead pupae (post-application, BFD +2aa to +23)		12	29	8	300
Daily mean flight intensity [foraging bees/m²/ replicate]	pre	10.3	8.3	8.4	10.7
	post (BFD +2aa)	19.0	12.2	15.0	15.3
	post (BFD +2aa to +9)	14.5	14.8	14.1	13.8

aa after application
pre average values for BFD to BFD +2ba (before application)
post (BFD +2aa) main application day after application
post (BFD +2aa to +9) mean value for BFD +2aa to BFD +9
post (BFD +2aa to +23) mean value for BFD +2aa to BFD +23
$Q_{M(average)}$ mean mortality per day after application divided by mean mortality per day before application
BFD brood area fixing day

Regarding the total daily adult bee mortality observed during the exposure of the bees to the BYI 08330 OD 100 treated plants (treatment group T1 and T2) in the tunnels and in the time afterwards no remarkable observations were made in the test item treatment compared to the control or toxic standard treatment. The mean post-application adult bee mortality was comparable in all treatment groups (T1: 5.5 dead bees/replicate/day, T2: 4.5 dead bees/replicate/day, control: 4.1 dead bees/replicate/day, toxic standard: 8.1 dead bees/replicate/day). In the toxic standard treatment, all three colonies showed an increased number of dead pupae in approximately the same period of time from BFD +12 to BFD +19 which is a typical effect of Insegar WG 25. The total number of dead pupae per colony from BFD +2aa to BFD +23 was 12 dead pupae in the test item treatment T1, 29 dead pupae in treatment T2, 8 dead pupae in the control treatment compared to 300 dead pupae in the toxic standard treatment.

B. Observations

<u>Honey bee flight intensity:</u>

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The application(s) of BYI 08330 OD 100 on the oil-seed spring rape and of the toxic standard had no effects on the flight intensity compared to the control treatment. The daily mean post-application (BFD +2aa to BFD +9) flight intensity was on a similar level in all treatment groups with 14.5 bees/m²/replicate in the test item treatment T1, 14.8 bees/m²/replicate in the test item treatment T2, 14.1 bees/m²/replicate in the control and 13.8 bees/m²/replicate in 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 item treatment groups T1 and T2 compared to the control treatment. The colonies of the test item treatment groups and control showed all brood stages at the assessment dates during the experimental phase of the study. In one colony of the toxic standard no eggs were observed and queen cells were noticed at the last brood assessment which is the evidence that the queen of this colony died.

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) showed the course to be expected in a natural bee development cycle in two colonies of the test item treatment group T1, in the colonies of treatment group T2, in two colonies of the control group and in one colony of the toxic standard. In one control colony and in one colony of T1 a termination of brood in single cells was observed during the observation period which resulted in a retarded increase of the brood index from BFD to BFD +9 compared to the other test hives of the same treatment. In the colonies of T2 about 25% of the marked cells showed a termination of the bee brood during the test. However, since one control colony showed a high termination rate between BFD and BFD +4 and the other two colonies of the control showed a termination of about 20% during the test, the effects noticed in the test item treatments can not be ascribed to the application of the test item. Colony 3R of the toxic standard treatment was obviously affected by the treatment with Insegar WG 25 as can be seen in the decreasing index between the first assessment and BFD +4, as well as in the very low index on the following assessment on BFD +9.

Honey bee behaviour in front of the colonies and within the crop area:
No differences regarding the behaviour of the bees were noticed between the test item treatment groups T1 and T2, the toxic standard treatment and the control.

CONCLUSION

It was concluded that the application of BYI 08330 OD 100 applied five times (treatment group T1) and once (treatment group T2) on a bee-attractive flowering crop such as oil-seed spring-rape did not result in an adverse effect on the mortality of adult honey bees and brood, flight intensity of the bees on the crop and the behaviour of the bees in the crop area or in front of the hive. An irritation of the brood development at the earlier assessments was noticed in one colony of the test item treatment group T1, and about one quarter of the marked cells showed a termination of bee brood in the colonies of treatment group T2. However, since one control colony showed a high termination rate between BFD and BFD +4 and the other two colonies of the control showed a termination of about 20% during the test, the effects noticed in the test item treatments can not be ascribed to the application of the test item. Whatever the cause, the colonies were in good condition during the observation period of 29 days.

([redacted] A., 2005)

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KIIIA1 10.4.7/02 → [redacted] (2007), M-294216-01-1, summary filed under KIIIA 10.4.4/01

Report:	**KIIIA1 10.4.7/03, [redacted], [redacted] C. & [redacted] J.; 2009**
Title:	Effects of Spirotetramat OD 150 (Movento®) on honeybees in a greenhouse trial in strawberries in Germany Date: 2009-09-01
Organisation:	Bayer CropScience AG, [redacted], Germany
Report No.:	SBJ-H9-2009, M-354707-01-1
Publication:	Unpublished
Dates of experimental work:	2009-04-13 to 2009-05-14
Guidelines:	EPPO 170 (3)
Deviations:	None
GLP:	No (but conducted under GEP)

Executive summary

The aim of the study was to examine the potential effects of Spirotetramat OD 150 on the honey bee (*Apis mellifera* L.) applied via spray application onto strawberries under greenhouse conditions.

Small honey bee colonies (approx. 3500 honey bees) were confined in tunnels (500 m²) placed on a strawberry field (variety Darselekt) in [redacted] (Nordrhein-Westfalen, Germany). Two replicates were set up for each treatment group, an untreated control, the test item and the toxic reference. The test item was applied once onto strawberries at 100 g a.s./ha in 400 L water. Assessments on the bees started 3 days before the application. The colonies were examined for test item-related effects for 11 days after the application (until DAT 11) inside the tunnels. The endpoints mortality, foraging activity, nectar and pollen storage, egg laying and breeding activity, colony strength and hive weight development were assessed. After removal of the hives from the tunnels further assessments were performed until DAT 28 (only brood, colony strength, hive weight, food stores). Insegar (containing fenoxycarb at nominally 250 g/kg, 150 g a.s./ha in 400 L) was used as toxic reference.

No difference in foraging activity and adult mortality was found in the pre-treatment and in the post-treatment period. An increased pupal mortality was observed in the toxic reference group in the post-treatment period. No test item-related differences were found in nectar and pollen storage, egg laying activity, larval and pupal abundance, colony strength and hive weight development between control, test item and toxic reference at study termination.

There were no test item related effects in any endpoint.

MATERIAL AND METHODS

A Materials

1. Test material	Spirotetramat OD 150
Description	Not stated
Lot/batch No.	2008-010865*0,5
Nominal content a.s.	150 g/L
Stability of test compound	Not stated
2. Vehicle and/or positive control	Positive control: Insegar (containing fenoxycarb at nominally 250 g/kg, 150 g a.s./ha in 400 L water, CAS-No. of a.s.: 79127-80-3)
3. Test animals	
Species	Honey bee (*Apis mellifera* L.)
Age	colonies with approx. 3500 adult bees
Source	Honey bee colonies with no signs of *Varroa* or *Nosema* infestation and queen-right, bred by a German beekeeper (M. [redacted], Germany)

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Environmental conditions	Plastic tunnels (500 m²) placed on a strawberry field
Temperature	Inside one tunnel: 4.7 – 42.5°C (min – max on different days)
Relative humidity	Inside one tunnel: 36.7 - 100% (min – max on different days)
Photoperiod	Natural daylight

B Study design and methods

1. In life dates: April 13, 2009 - May 14, 2009

2. Experimental treatments

Test units were plastic tunnels (5 m x 100 m) placed on the strawberry field prior to the application. For each treatment group (control, test item and toxic reference) two replicate tunnels were set up. The bee colonies were placed inside the tunnels during flowering of the crop.

Control: Control plants remained untreated.

Test item: Nominal application rate of the test item was 100 g a.s./ha.

Toxic reference item: Nominal application rate of the toxic reference was 150 g fenoxycarb/ha.

Application of the test item in the tunnel test:

The application was performed at BBCH 65, when 50% open blossoms on 50% of the individual strawberry plants present in the tunnels were visible. A Schachtner sprayer with 5 flat fan nozzles was used, operated by compressed air at 2.0 bar. The water volume rate of 400 L/ha was used, resulting in 20 L water per tunnel. The spray lance was held approximately 40 cm above the crop while walking and spraying.

3. Observations

Assessments on the bees started 3 days before the application. The bee colonies were examined for test item-related effects for 11 days after the application (until DAT 11) inside the tunnels. In particular, the endpoints mortality, foraging activity, nectar and pollen storage, egg laying and breeding activity, colony strength and hive weight development were assessed. The assessed endpoints were compared between control, test item and toxic reference, and, within the test item treatment, between pre- and post application. After removal of the hives from the tunnels further assessments were performed until DAT 28 (only brood, colony strength, hive weight, food stores).

Results and Discussion

A. Findings

Effects of Spirotetramat OD 150 in strawberries on small honeybee colonies

Testing endpoint	Control	Test Item	Toxic Reference
	[average per treatment group]		
Foraging activity **ba** [Average No. of bees / m²]	7.2	7.3	7.3
Foraging activity DAT 0 **aa** [Average No. of bees / m²]	8.1	9.2	7.8
Foraging activity DAT 1 to DAT 11 [Average No. of bees / m²]	18.7	19.7	17.3
Foraging activity DAT 0 **aa** to DAT 11 [Average No. of bees / m²]	17.8	18.8	16.5
Adult mortality in front of hive **ba** [Total No. of dead bees]	6.5	16.5	2.0
Adult mortality in front of hive **aa** [Total No. of dead bees]	33.5	34.5	28.0
Adult mortality at tunnel edges **ba** [Total No. of dead bees]	32.0	78.0	85.5

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Testing endpoint		Control	Test Item	Toxic Reference
		[average per treatment group]		
Adult mortality at tunnel edges **aa** [Total No. of dead bees]		124.5	135.0	125.5
Pupal mortality in front of hive **ba** [Total No. of dead pupae]		2.0	0.5	0.5
Pupal mortality in front of hive **aa** [Total No. of dead pupae]		9.0	13.0	54.5
Hive weight development from DAT 0 until end of the study (DAT 28) [%]		+25.3	+25.1	+10.6
Nectar stores at study start and study end [cm² comb area]	DAT 0	392	227	446
	DAT 4	887	516	866
	DAT 7	846	464	701
	DAT 11	660	474	763
	DAT 14	289	248	248
	DAT 20	639	846	825
	DAT 28	413	454	557
Pollen stores at study start and study end [cm² comb area]	DAT 0	194	223	231
	DAT 4	194	136	177
	DAT 7	206	136	388
	DAT 11	219	111	326
	DAT 14	21	41	103
	DAT 20	50	141	186
	DAT 28	210	375	268
Egg-laying activity [cm² comb area with egg cells]	DAT 0	392	598	652
	DAT 4	301	297	330
	DAT 7	652	421	549
	DAT 11	561	392	586
	DAT 14	83	206	124
	DAT 20	557	639	1093
	DAT 28	392	186	516
Larval abundance [cm² comb area with uncapped cells]	DAT 0	363	780	908
	DAT 4	611	528	524
	DAT 7	371	466	66
	DAT 11	912	450	276
	DAT 14	215	351	619
	DAT 20	813	1526	1539
	DAT 28	578	454	639
Pupal abundance [cm² comb area with capped cells]	DAT 0	1135	1856	1444
	DAT 4	1196	1774	1815
	DAT 7	1444	1836	1815
	DAT 11	1073	1423	1044
	DAT 14	454	557	474
	DAT 20	1073	1238	672
	DAT 28	1712	2991	2434
Total abundance of brood [cm² comb area with brood cells]	DAT 0	1889	3234	3003
	DAT 4	2108	2599	2669

Testing endpoint		Control	Test Item	Toxic Reference
		[average per treatment group]		
	DAT 7	2467	2723	2430
	DAT 11	2545	2265	1906
	DAT 14	751	1114	1217
	DAT 20	2442	3404	3305
	DAT 28	2682	3630	3589
Colony strength [cm² comb area covered with adult bees]	DAT 0	928	1031	1073
	DAT 4	1176	1341	1258
	DAT 7	1712	1856	1877
	DAT 11	1980	2372	1980
	DAT 14	578	949	949
	DAT 20	1650	2537	2269
	DAT 28	1733	2826	1898

DAT: days after application **ba**: before the application **aa**: after application R: replicate TG: treatment group

B. Observations

In the pre-treatment, as well as in the post-treatment period no difference in foraging activity was found between control, test item and toxic reference.

Adult mortality was comparable in the pre-treatment, as well as in the post-treatment period for the control, test item and toxic reference.

Pupal mortality was comparable in the pre-treatment period for the control, test item and toxic reference. In the post-treatment period comparable numbers of dead pupae was found in the test item replicates as compared to the control. However, in the toxic reference group, an increased number of dead pupae was observed.

No test item-related differences were found in nectar and pollen storage, egg laying activity, larval and pupal abundance, colony strength and hive weight development between control, test item and toxic reference at study termination.

CONCLUSION

The results of the study show that there is no risk to honeybees by foraging on strawberries sprayed once with Spirotetramat OD 150 (Movento®) (application rate 100 g a.s./ha) under greenhouse conditions. There were no test item related effects in any endpoint.

([redacted] et al., 2009)

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Report:	**KIIIA1 10.4.7/04, [redacted] H.M.; 2010**
Title:	Effects of an application of Spirotetramat SC 100 to flowering raspberries on honeybee (*Apis mellifera*) brood in a semi-field study Date: 2010-05-20
Organisation:	[redacted], United Kingdom Bayer CropScience AG, [redacted], Germany
Report No.:	S3XZ1000; M-369450-01-1
Publication:	Unpublished
Dates of experimental work:	May 29, 2009 to June 26, 2009
Guidelines:	OECD Guidance document No. 75: Guidance Document on the Honey Bee (*Apis Mellifera* L.) Brood Test Under Semi-field Conditions
Deviations:	Not specified
GLP:	Yes

Executive summary

The aim of the study was to assess the effects of Spirotetramat SC 100 on honey bee workers and brood (*Apis mellifera* L.) when applied to flowering rasperries within a bee-proof tunnel.

Apis mellifera colonies in bee-proof tunnels were exposed for 16 days to a single dose of nominally 100 µg a.s./ha (Spirotetramat SC 100) followed by 8 days observation period. The test item was sprayed onto flowering raspberries in tunnels. Treatment group, water control and positive control consisted of 3 tunnels each. Mortality, foraging activity and behaviour at hive were assessed daily. Colony assessments were performed every 3-5 days. Samples of flowers, pollen and nectar from colonies were taken for residue analysis. Insegar (containing fenoxycarb at nominally 150 g a.s./ha) was used as toxic reference (positive control).

Application of the test item to flowering raspberries in tunnels resulted in no biologically significant increase in adult worker mortality when compared with the control. The test item showerd no apparent effect on foraging activity and on behaviour at the hive. Only the development of cells marked at the egg stage was affected by the test item.
Early stages of honeybee larval development were more sensitive to the effects of Spirotetramat SC 100 exposure than later stages.

Material and Methods

A Materials

1. Test material	Spirotetramat SC 100 (trade name: Movento)
Description	Suspension concentrate, white liquid
Lot/batch No.:	Batch No. 2007-005473
Analytical content a.s.	9.33% w/w
Stability of test compound	Test item is considered stable under test conditions. Expiry date: 2010-02-16, when stored at +20 °C ± 5 °C
2. Positive control	<u>Positive control</u>: Insegar WG (containing fenoxycarb, nominal content of a.s.: 25% w/w, batch No.: SM08 B302 REL 02/08)
3. Test animals	
Species	Honey bee (*Apis mellifera* L.)
Age	Colonies including immature and adult stages
Source	standardised and queen-right honey bee field colonies, low incidence of minor brood disease (chalkbrood, sacbrood and baldbrood), American foulbrood (*Paenibacillus larvae* subsp. *larvae*) and European foulbrood (*Melissococcus plutonius*) were clinically

	absent, the presence of *Varroa destructor* was monitored, all test colonies headed by newly mated queens (apart from one colony), obtained from the Fera National Bee Unit colonies
Environmental conditions	Bee-proof tunnels (30 m x 6 m, roof area covered with polythene and the sides with insect-proof mesh) containing flowering raspberries
Temperature	Measurements were taken throughout the trial using a TinyTag datalogger (see Appendix 1 of the report)
Relative humidity	Measurements were taken throughout the trial using a TinyTag datalogger (see Appendix 1 of the report)
Photoperiod	Length of the days

B Study design and methods

1. Experimental dates — May 29, 2009 - June 26, 2009

2. Experimental treatments

Test units were 9 test tunnels of 30 m x 6 m with the roof area covered with polythene and the sides with insect-proof mesh to contain the bees. The tunnels were planted with 4 rows of raspberries (variety Glen Ample) under similar conditions to normal commercial practice (20-25 cm between plants within the rows, 1-1.5 m between rows). The paths between the rows along the length of the tunnel were covered with sheetings, in order to facilitate the collection of dead bees. One colony was used per test unit. The colonies were transferred from their holding apiary, where they were allowed to fly freely, into the tunnels 4 to 5 days before the application. Each colony was provided with a limited amount of stores to ensure feeding on the crop is encouraged. A supply of clean water was provided.

Control: Three control tunnels received no chemical treatment. These tunnels were sprayed with water in a volume of 1000 - 1200 L/ha, i.e. to run-off.

Test item: Test item was applied at nominal 100 g a.s./ha to 3 tunnels in a volume of 1000 – 1200 L/ha, i.e. to run-off.

Toxic reference item: Insegar (fenoxycarb) was applied to 3 tunnels at 150 g a.s./ha in a volume of 1000 – 1200 L/ha, i.e. to run-off.

Application of the test item in the contact test:

The application was preceded by the first foraging assessment of the day. The application was made using a backpack sprayer with a single spray nozzle (Hardi ISOF110). Each row in a tunnel was sprayed individually, with the spray jet being turned on at the beginning of the row and off at the end. The spray was applied from one side but the spray operator ensured even coverage of both sides of the row. One set of equipment was used to spray the water and toxic reference and a second to apply the test item.

3. Observations

Numbers of dead bees on sheets within crop and dead bee traps were counted daily. Colony assessments were performed every 3-5 days to determine levels of bees, sealed and unsealed brood, stores and pollen. Brood cells containing eggs, 1-2 days old larvae or 3-4 day old larvae (100 of each) were marked in each colony on day prior to spray application and contents assessed every 3-5 days. Samples of flowers, pollen and nectar from colonies were taken for residue analysis.

The exposure duration was 16 days followed by 8 days observation period.

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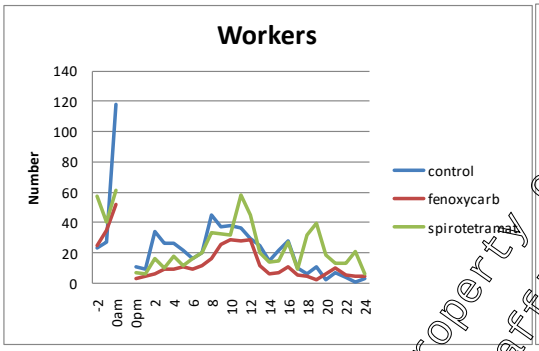
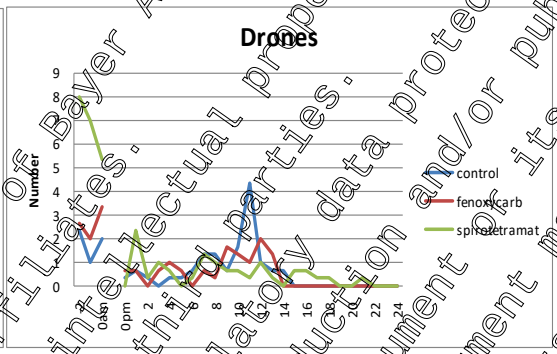
RESULTS AND DISCUSSION

A. Findings

The mean numbers of dead bees collected in the control, fenoxycarb and Spirotetramat SC 100 treated tunnels are shown in Figure 1 below.

Figure 1: Numbers of dead workers, drones and pupae/larvae found in the tunnels treated with water (control), Spirotetramat SC 100 or fenoxycarb





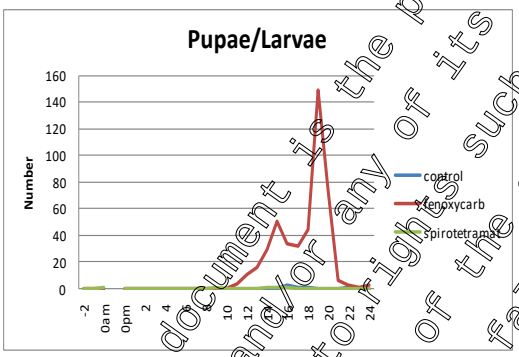
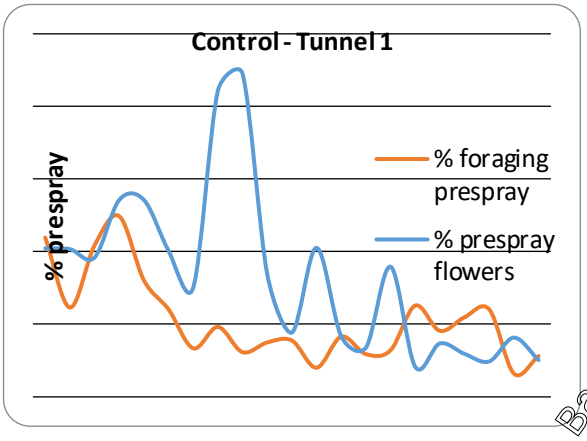
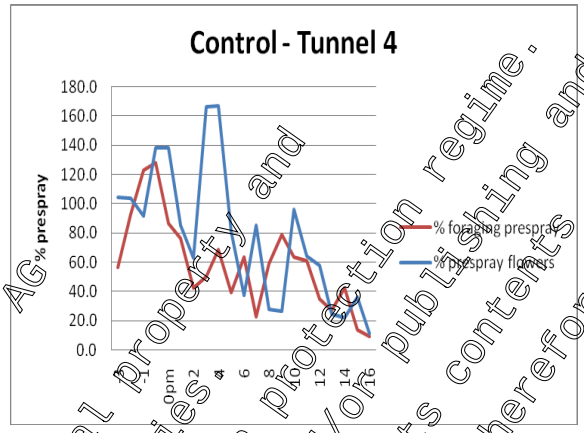
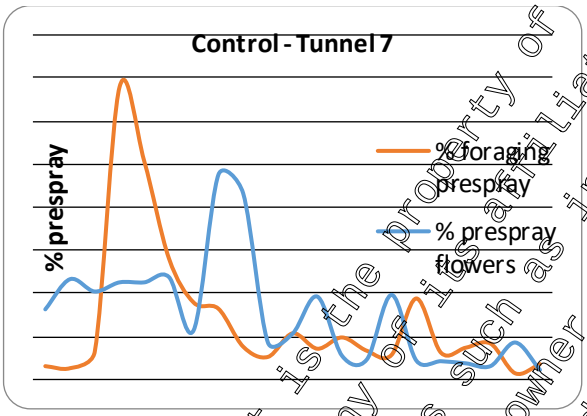


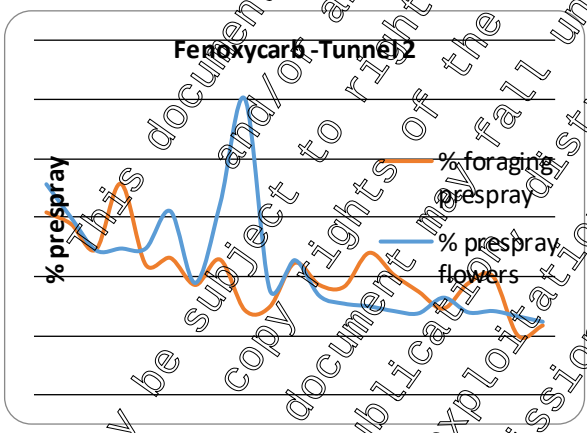
Figure 2 shows the foraging activity over the period that the bees were in the tunnels compared with the flowers present as a percentage of pre-spray.

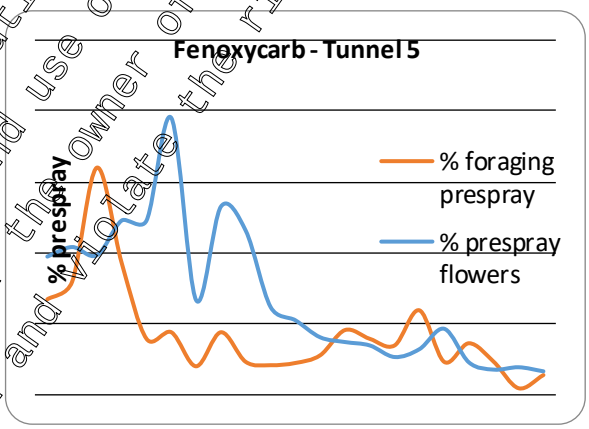
Figure 2: **Relative foraging and flowering in each tunnel expressed as a percentage of pre-spray levels (mean day-3 to day of application).**

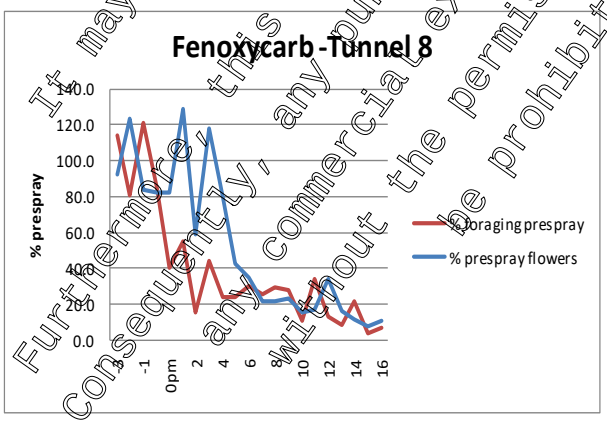


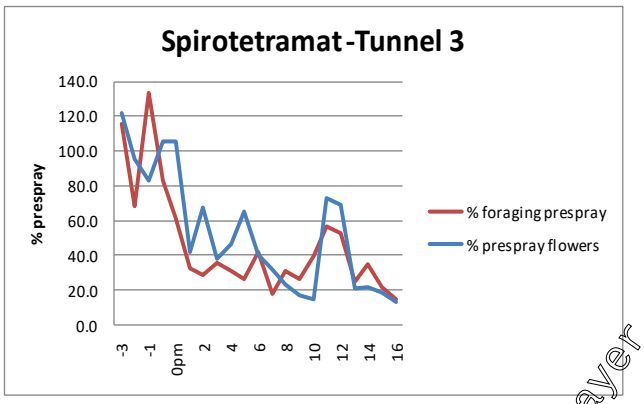


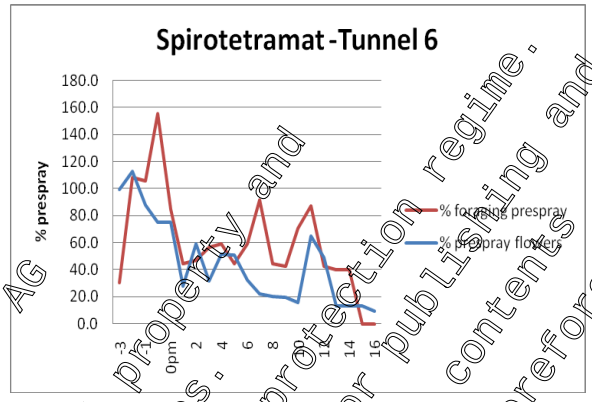


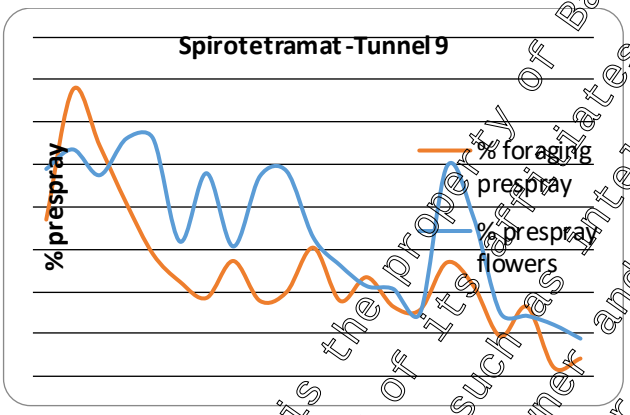






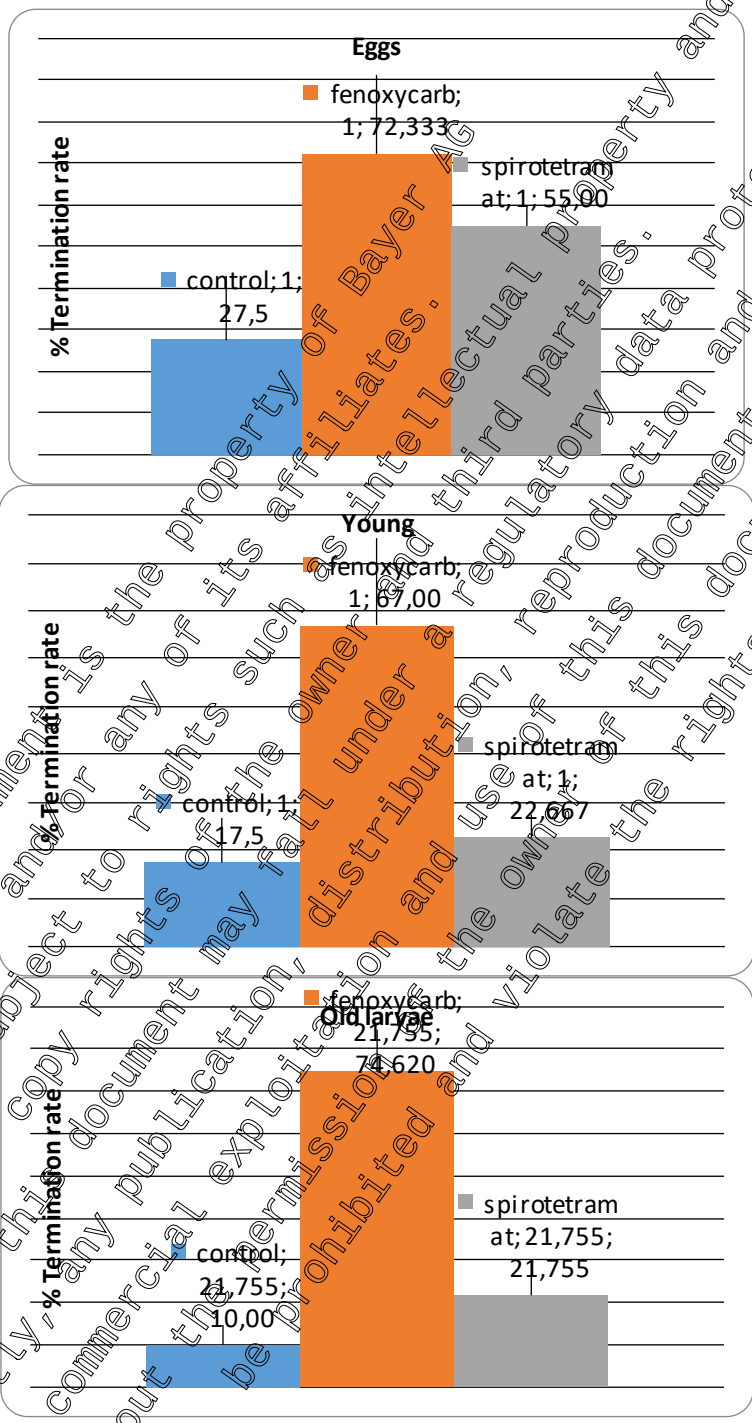






Detailed assessments of the brood termination rate are summarised in Figure 3 below.

Figure 3: Brood termination rate for eggs, [redacted] larvae and old larvae marked in colonies in control, fenoxycarb and Spirotetramat SC 100 treated tunnels



B. Observations

Application of Spirotetramat SC 100 (Movento) to flowering raspberries in tunnels at 95-104 g a.s./ha resulted in no biologically significant increase in adult worker mortality when compared to the control. Application of the toxic reference fenoxycarb resulted in large numbers of pupae/larvae being observed dead within the tunnels.

Neither Spirotetramat SC 100 nor fenoxycarb showed any apparent effects on foraging activity when compared with control tunnels and there were no apparent effects on behaviour at the hive.

Assessments of the brood within the colony in each tunnel showed that fenoxycarb exposure resulted in high termination rates for developing eggs (72%), [redacted] larvae (67%) and old larvae (75%). Spirotetramat SC 100 exposure resulted in a 55% termination rate of the marked eggs (control 28%) but termination rates for [redacted] larvae and old larvae were 21-23% and were closer to that of controls (18% [redacted] larvae, 10% old larvae). The Brood Index data showed that the development of cells marked as eggs, [redacted] and old larvae were affected by fenoxycarb whereas only the development of cells marked at the egg stage was affected by Spirotetramat SC 100. These data were supported by the full colony assessments which showed a decline in the levels of unsealed brood within the Spirotetramat SC 100 exposed colonies resulting in the delayed decrease in the levels of sealed brood compared with control colonies.

Conclusion

Exposure of honeybee colonies to Spirotetramat SC 100 resulted in effects at the early stages of larval development with termination of development in 55% of cells marked as containing eggs prior to exposure. There was less termination of development observed in cells marked as [redacted] larvae (1-2 days after hatching) or old larvae (3-5 days after hatching).

([redacted] H.M., 2010)

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IIIA1 10.5 Effects on arthropods other than bees

Table IIIA1 10.5-1: Ecotoxicological endpoints for arthropods other than bees exposed to Spirotetramat OD 150

Test Species / Reference	Test Substance	Exposure	Application Rate	Ecotoxicological endpoint
Parasitoids				
Aphidius rhopalosiphi KIIA 8.8.1.1/01	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 KIIA 8.8.2.1/01	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 KIIA 8.8.1.2/01	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 KIIA 8.8.2.2/01	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
Typhlodromus pyri KIIIA1 10.5.2/01	OD150	Aged residues., potted apple trees	4 × 72 g a.s./ha	58.3, 50.5 and 10.8% corr. mortality were found on DAA 42, 49 and 56. After 7 weeks of aging, no effects > 11% on survival or reproduction were observed
Mite fauna KIIIA1 10.5.4/01	OD150	Field test in vineyards	2 x 96 g a.s./ha 1 x 36.4 & 1 x 35 g a.s./ha 2 x 9.1 g a.s./ha 4 x 4.8 g a.s./ha	No adverse effects on the acarine mite fauna
Foliage-dwelling predators				
Chrysoperla carnea KIIA 8.8.2.4/01	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 KIIA 8.8.2.4/02	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

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Risk assessment procedures
In general the evaluation of the studies with non-target arthropods is based on the ESCORT 2 trigger of concern of 50% effects at the maximum application rate. In addition, the following publications are also taken into consideration.

- CANDOLFI *et al.*: Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods; ESCORT 2 workshop (European Standard Characteristics of Non-Target Arthropod Regulatory Testing), Wageningen, NL, March 21-23, 2000, SETAC Europe; SETAC publication August 2001
- Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329/2002, rev 2 final, 17 October 2002

ESCORT 2: Hazard Quotient (HQ) calculation
The hazard quotient calculations were conducted according to the guidance document of the ESCORT 2 workshop (published 2001).
The following equations were used to calculate the HQ values with both indicator species, for in-field and off-field exposure scenarios, respectively:

$$\text{In-field HQ} = \frac{\text{Max. in - field rate}}{LR_{50}} \qquad \text{Off-field HQ} = \frac{\text{Max. off - field rate}}{LR_{50}}$$

Triggers:
Tier 1: The risk for in-field or off-field is considered acceptable if the HQ values for both indicator species are <2, or the effects in limit tests are <50%.
Tier 2: The risk is considered acceptable if effects are <50%.

Potential exposure
The exposure scenario is based on the use pattern as given in Table IIIA1 10.1.

in-field
The max. in-field rate (maximum residue on soil or leaf surface respectively) has been calculated according to the following formula:

in-field rate = max. single application rate × MAF (× corr. factors 3 dim. cultures)

- MAF = **M**ultiple **A**pplication **F**actor: was determined to estimate the influence of the repeated application on predicted environmental concentration (PEC), according to the following equation:
  $MAF = (1-e^{-ni})/(1-e^{-ki})$
  $k = \ln(2)/DT_{50}$
  n = number of applications
  i = interval between the applications [d]
- For spirotetramat, a DT_{50} of 3.42 days was reported in [redacted] et al. 2006 (2006, KIIIA1 10.1.2/01)
- Correction factor 3-dimensionale cultures = 0.5 (according to ESCORT 2, the application rate for orchard and vineyard applications is multiplied by a correction factor of 0.5 for 3-dimensional crops for in-crop PEC calculation.)

Table IIIA1 10.5-2: In-crop exposure for non-target arthropods and Spirotetramat OD 150

Crop/ No. of applications/ application interval [d]	Appl. rate [g a.s./ha]	MAF	Corr. factor 3-dimens. cultures	Max. in-field rate [g a.s./ha]
Fruit crops (orchards)/ 2 / 21	96*	1.01**	0.5	48.5
Vegetables (lettuce)/ 2 / 14	72	1.06**	-	76.3

* For the calculation of exposure of leaf-dwelling arthropods, it is appropriate to use the application rate related to 1 m canopy height rather than the 288 g a.s./ha for 3 m canopy height. In a 3-dimensional crop system, the applied substance will be evenly distributed in the applied canopy. For a higher canopy, a higher substance amount is needed to cover every individual leaf with the respective substance amount, and in so far the substance residue level per leaf will be the same after an application of the single rate to 1 m canopy and after the three-fold amount to 3 m canopy.

* based on a DT_{50} of 3.42 days

off-field

The max. off-field rate (maximum residue on the soil leaf surface respectively) has been calculated according to the following formula:

$$Off\text{-}field\ rate = maximum\ \ application\ rate \times MAF \times \frac{drift\ factor}{vegetation\ \ distribution\ factor} \times correction\ factor$$

- MAF = explanation see above (calculation of in-field rate)
- drift factor = The calculations of the off-crop exposure rates are based on the drift rates as published in Ganzelmeier et al., 2000.
- Vegetation distribution factor = 10 (assumed standard value to adapt the overestimated exposure given by the 90th percentile drift values to a more realistic deposit estimation for off-field habitats; in case the ecotoxicological endpoint is derived from a 2-dimensional test system, a vegetation distribution factor is employed to account for the 3-dimensional structure of the off-field vegetation)
- Correction factor = 5 (uncertainty factor for the extrapolation from indicator species to all off-field non-target arthropods). Since laboratory as well as extended laboratory data are available for a range of species, the factor is set to 5.

Table IIIA1 10.5-3: Off-crop exposure for non-target arthropods and Spirotetramat OD 150

Crop/ No. of applications/ application interval [d]	Appl. rate [g a.s./ha]	MAF*	Drift [%] (distance)	Veg. distr. factor	Corr. factor	Max. off-field rate [g a.s./ha]
Fruit crops (orchards) / 2 / 21	288	1.01	12.13 (3 m) **	10	5	17.6
Vegetables (lettuce) / 2 / 14	72	1.06	2.38 (1 m)	10	10 (Tier 1)	1.82
					5 (Tier 2)	0.91

* based on a DT_{50} of 3.42 days

** drift rate for late application, since application in citrus is only to foliate trees

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Endpoint used in the risk assessment:
In the first tier the risk assessment is conducted based the endpoints determined in laboratory studies with organisms exposed to Spirotetramat OD 150 sprayed onto on glass plates. The tests resulted in a LR_{50} of 114.7 g a.s./ha for *Aphidius rhopalosiphi* and in a LR_{50} of 0.333 g a.s./ha for *Typhlodromus pyri*. In the second tier the risk assessment is based on the endpoints of the extended laboratory tests with *Aphidius rhopalosiphi* exposed to Spirotetramat OD 150 on barley plants, and *Chrysoperla carnea* and *Coccinella septempunctata* exposed to Spirotetramat OD 150 on bean leaves. All studies resulted in LR_{50} values of >288 g a.s./ha. The most sensitive species was *Typhlodromus pyri*, for which in an extended laboratory study the LR_{50} of 1.588 g a.s./ha was determined after exposure to Spirotetramat OD 150 sprayed onto bean leaves (KIIA 8.8.2.2/01).

Tier 1 risk assessment
The standard species for the Tier 1 risk assessment are the parasitoid *Aphidius rhopalosiphi* and the predatory mite *Typhlodromus pyri* (SANCO/10329/2002-final). For the hazard calculation results of the laboratory studies with these indicator species exposed to Spirotetramat OD 150 are considered.

Within-field environment

Table IIIA1 10.5-4: HQ calculation for the in-field and the individual LR_{50} determined on glass plates with *Aphidius rhopalosiphi* and *Typhlodromus pyri* exposed to Spirotetramat OD 150

Crop / max. no. of applications / min. application interval	Indicator species	LR_{50} [g a.s./ha]	in-field rate [g a.s./ha]	HQ	ESCORT 2 HQ trigger	Refined risk assessment needed?
Orchards / 2 / 21	*Aphidius rhopalosiphi*	114.7	48.5	0.4	< 2	**No**
Lettuce / 2 / 14			76.3	0.7		
Orchards / 2 / 21	*Typhlodromus pyri*	0.333	48.5	146	< 2	**Yes**
Lettuce / 2 / 14			76.3	229		

Since the ESCORT 2 HQ trigger of 2 was not passed for *T. pyri* in the Tier 1 risk assessment, a higher-tier risk assessment is provided for this species based on extended laboratory data. In this higher-tier risk assessment, also *Coccinella septempunctata* and *Chrysoperla carnea* are included for which only extended laboratory tests were conducted.

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Tier 2 risk assessment

Table IIIA1 10.5-5: HQ calculation for the in-field and the individual LR_{50} determined under extended laboratory conditions with *A. rhopalosiphi* and *T. pyri, C. carne* and *C. septempunctata* exposed to Spirotetramat OD 150

Crop / max. no. of applications / min. application interval	Indicator species	LR_{50} [g a.s./ha]	in-field rate [g a.s./ha]	HQ	ESCORT 2 HQ trigger	Refined risk assessment needed?
Orchards / 2 / 21	*Aphidius rhopalosiphi*	> 288	48.5	< 0.2	< 2	**No**
Lettuce / 2 / 14			76.3	< 0.3		
Orchards / 2 / 21	*Typhlodromus pyri*	1.588	48.5	30.5	< 2	**Yes**
Lettuce / 2 / 14			76.3	48.0		
Orchards / 2 / 21	*Chrysoperla carnea*	> 288	48.5	< 0.2	< 2	**No**
Lettuce / 2 / 14			76.3	< 0.3		
Orchards / 2 / 21	*Coccinella septempunctata*	> 288	48.5	< 0.2	< 2	**No**
Lettuce / 2 / 14			76.3	< 0.3		

Since the ESCORT 2 HQ trigger of 2 was not passed for *T. pyri* in the 2nd Tier risk assessment, another higher-tier risk assessment is provided for this species based on aged-residues and field data.

Higher tier risk assessment (in-field)

Typhlodromus pyri

To investigate the effects of Spirotetramat OD 150 to predatory mites under realistic conditions, a field study was conducted in a vineyard (KIIIA1 10.5.4/01). A realistic use scenario of 2 x 96 g a.s./ha was applied[13], covering the citrus as well as the lettuce use scenario discussed here. In this study, no adverse effects to predatory mite populations were found in none of the assessments conducted.

The lettuce scenario is as well covered by an aged-residues study with *T. pyri*, in which in a realistic application scenario 4 x 72 g a.s./ha were applied. In this study, no adverse effects to the test organisms were observed from seven weeks onwards. In so far, the potential for recovery within one season was shown.

It can thus be concluded that the application of Spirotetramat OD 150 as referred to here will not pose unacceptable effects to populations of sensitive arthropod taxa in in-crop habitats.

[13] The single application rate of 96 g a.s./ha in this field study in a vineyard has to be considered to cover the citrus application rate as well, which is 96 g a.s. per ha and m canopy height, i.e. at max. 288 g a.s./ha with 3 m canopy height. The field study referred to here was done in a model crop with at maximum 1 m canopy height. In higher crops, the increased substance amount applied is evenly distributed in the increased foliage volume which is growing along with canopy height. This means that after an application the substance concentration on a leaf or a blossom will be the same irrespectively whether a 1 m high crop was applied with 96 g a.s./ha or a 3 m high crop was applied with the three-fold substance amount of 288 g a.s./ha. Thus, the exposure per three-dimensional volume unit of the crop system is the same irrespectively of the canopy height of the treated crop and the accordingly adjusted hectare-application rate.

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Off-field environment

Tier 1 risk assessment

Table IIIA1 10.5-6: HQ calculation for the off-field and the individual LR_{50} determined on glass plates with *Aphidius rhopalosiphi* and *Typhlodromus pyri* exposed to Spirotetramat OD 150

Crop / max. no. of applications / min. application interval	Indicator species	LR_{50} [g a.s./ha]	off-field rate [g a.s./ha]	HQ	ESCORT 2 HQ trigger	Refined risk assessment needed?
Orchards / 2 / 21	*Aphidius rhopalosiphi*	114.7	17.6	0.15	< 2	No
Lettuce / 2 / 14			1.82	0.02		
Orchards / 2 / 21	*Typhlodromus pyri*	0.333	17.6	53	< 2	Yes
Lettuce / 2 / 14			1.82	5.5		

Since the ESCORT 2 HQ trigger of 2 was not passed for *T. pyri* in the 1st Tier risk assessment for the off-crop environment, a higher-tier risk assessment is provided for this species based on extended laboratory data. In this higher-tier risk assessment, also *Coccinella septempunctata* and *Chrysoperla carnea* are included for which only extended laboratory tests were conducted.

Tier 2 risk assessment

Table IIIA1 10.5-7: HQ calculation for the off-field and the individual LR_{50} determined under extended laboratory conditions with *A. rhopalosiphi* and *T. pyri, C. carne and C. septempunctata* exposed to Spirotetramat OD 150

Crop / max. no. of applications / min. application interval	Indicator species	LR_{50} [g a.s./ha]	off-field rate [g a.s./ha]	HQ	ESCORT 2 HQ trigger	Refined risk assessment needed?
Orchards / 2 / 21	*Aphidius rhopalosiphi*	> 288	176*	< 0.6	< 2	No
Lettuce / 2 / 14			9.1*	< 0.03		
Orchards / 2 / 21	*Typhlodromus pyri*	1.588	17.6	11.1	< 2	Yes
Lettuce / 2 / 14			0.91	0.6		No
Orchards / 2 / 21	*Chrysoperla carnea*	> 288	17.6	< 0.06	< 2	No
Lettuce / 2 / 14			0.91	< 0.003		
Orchards / 2 / 21	*Coccinella septempunctata*	> 288	17.6	< 0.06	< 2	No
Lettuce / 2 / 14			0.91	< 0.003		

*Since the study has been conducted in a 3-Dim-test design, a vegetation distribution factor is not applied.

Since the ESCORT 2 HQ trigger of 2 was not passed for *T. pyri* and application in citrus orchards in the 2nd Tier risk assessment, another higher-tier risk assessment is provided for this species based on more realistic field data.

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Higher tier risk assessment (off-field)

In a field study in a vineyard, the effects of applications of Spirotetramat OD 150 to the predatory mite fauna were investigated (KIIIA1 10.5.4/01). In this study, the full citrus rate was applied as well as several drift rates referring to the citrus as well as to the vegetables scenario. Neither at the full rate nor at any of the drift rates significant effects to the mite fauna were seen[14].

It can hence be concluded that applications of Spirotetramat OD 150 according to application scenarios as under evaluation here will not cause unacceptable adverse effects to populations of sensitive arthropod taxa in off-crop habitats.

IIIA1 10.5.1 Effects on sensitive species already tested, artificial substrates

See KIIA 8.8.1.1/01 and KIIA 8.8.1.2/01

IIIA1 10.5.2 Effects on non-target terrestrial arthropods in ext. laboratory tests

See KIIA 8.8.2.1/01, KIIA 8.8.2.2/01, KIIA 8.8.2.4/01 and to KIIA 8.8.2.4/02

Report:	**KIIIA1 10.5.2/01, [redacted], A.; 2006**
Title:	BYI 08330 150 OD: Toxicity to the predatory mite *Typhlodromus pyri* SCHEUTEN (Acari, Phytoseiidae) using an extended laboratory test (under semi-field conditions aged residues on apple trees). Code: AE 1302943 00 OD15 A101 Date: 2006-01-18
Organisation:	Bayer CropScience GmbH, [redacted], Germany
Report No.:	CW05/028
Publication:	unpublished
Dates of experimental work:	May 03, 2005 – August 02, 2005
Guidelines:	IOBC(Blümel et al. 2000)
Deviations:	The test design was modified - potted apple trees were treated instead of glass plates, and mites exposed to freshly applied and under semi-field conditions aged residues on excised apple leaf discs
GLP	yes (certified laboratory)

Material and methods

Test item: Spirotetramat (BYI 08330) 150 OD, analytical content of spirotetramat: 148.89 g/L, specified by sample no. TOX07034-00, batch no.: 08030/0189(0152) and product code: AE 1302943 00 OD15 A101

Test organisms: Predatory mites *Typhlodromus pyri*

The aim of the study was to determine the toxicity of the test item to the predatory mite *Typhlodromus pyri* in an extended laboratory test after residual contact exposure to under semi-field conditions aged residues on potted apple trees, and the duration of effects after the application. BYI 08330 150 OD was applied with 4 x 72 g a.s./ha in 600 L water/ha on potted apple trees. The control was treated with deionised water in the same way as the test item group. The toxic reference dimethoate was applied at each bioassay date under extended laboratory conditions. Aging of the spray residues on the potted apple trees took place under natural semi-field conditions with rain protection during the whole study.

[14] One exception to this is the second assessment of the treatment group with the lowest treatment rate (4 x 4.8 g a.s./ha) where a statistically significant difference in mite abundance was seen compared to the control. However, this finding is clearly erratic and cannot be explained by a control effect, since in all other assessments for this and all the other treatment groups, in which considerably higher rates were applied, no effects at all were consistently detected. For further explanatory notes of this finding, see study summary KIIIA1 10.5.4/01.

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Bioassays were initiated on DAA (days after last test substance application) 0; 7; 14; 21; 28; 42; 49 and 56: leaves were taken from the apple trees, and predatory mites were exposed on these leaves under laboratory conditions. In each bioassay, mortality of 100 protonymphs of *T. pyri* was assessed over a period of 7 days after exposure on excised leaf discs by counting the number of living and dead mites for each bioassay. 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 by counting the total number of offspring (eggs and larvae) produced. From these data the endpoints mortality and effects on reproduction were calculated.

Findings
The results are considered as valid since the mortality/escaping value for the control stated in the laboratory method on glass was reached in this study (< 20%) 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.

The mean corrected mortality of the nymphs, and the mean reproduction rate of the surviving females exposed to the test item and the toxic reference is given below:

Effects on mortality

Test item	Spirotetramat 150 OD							
Test object	*Typhlodromus pyri*							
Exposure	Dried spray deposits on apple leaf discs							
Days after last application	0	7	14	21	28	42	49	56
Treatment	Mortality after 7 days (%)							
Control	5	4	5	17	6	4	3	7
	Corr. Mortality [%]							
Test item	93.7	100	90.5	92.8	100	58.3	50.5	10.8
Reference item (DAA1)	100	100	100	100	100	100	100	100

Effects on reproduction

Test item	Spirotetramat 150 OD		
Test object	*Typhlodromus pyri*		
Exposure	Dried spray deposits on apple leaf discs		
Treatment	Control	Test item	
	Mean no. of eggs /female	Mean no. of eggs /female	Reduction rel. to control [%]
DAA 0 to day 42	n.d.	n.d.	n.d.
DAA 49	5.19	5.94	-14.48
DAA 56	6.88	7.87	-14.35

n.d.: not determined
DAA: days after last application

Conclusions
In this extended laboratory test the lethal and sublethal effects of Spirotetramat OD 150 residues (aged under semi-field conditions) on the predatory mite *Typhlodromus pyri* were determined after application of 4 x 72 g a.s./ha onto apple trees. 58.3, 50.5 and 10.8% corr. mortality were found on DAA 42, 49 and DAA56. After 7 weeks of aging, no effects > 11% on survival or reproduction were observed any longer.

IIIA1 10.5.3 Effects on non-target terrestrial arthropods in semi-field tests

No semi-field test has been conducted since a field test is available (see KIIIA1 10.5.4/01).

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IIIA1 10.5.4 Field tests on arthropods species

Report:	**KIIIA1 10.5.4/01, [redacted], S.; 2006**
Title:	Evaluating effects of BYI 08330 OD 150 applications on mite fauna (Acari) in the field (grape vines, France). Date: 2006-03-14
Organisation:	[redacted], The Netherlands Bayer CropScience GmbH, [redacted], Germany
Report No.:	B127AFG; M-269107-01-1
Publication:	unpublished
Dates of experimental work:	June 08, 2005 – September 22, 2005
Guidelines:	Blümel *et al.*, 2000; Candolfi *et. al.*, 2000
Deviations:	no
GLP	yes (certified laboratory)

Material and methods

Test item: Spirotetramat (BYI 08330) OD 150, purity: BYI 08330: 148.89 g a.s./L (150 g a.s./L, nominal), density 0.986 g/mL, specified by sample no. TOX07034-00, batch no. 08030/0189(0152) and development no. 30-00364846, stable until 2006-03-10

BYI 08330 OD 150 is an insecticide proposed for use in a variety of crops, among these vineyards. Potential side-effects of this test item for phytoseiid mites were tested in a field study. The study was performed in a commercial vineyard in [redacted] in the Armagnac region, South-West of France. The study design was based on internationally acknowledged guidelines (Blümel et al., 2000; Hassan, 1985; Sterck and Vanwetswinkel, 1988; Boller et al., 1988; Englert et al., 1991, Candolfi et. al., 2000).

Four spray scenarios of BYI 08330 OD 150 were tested, as indicated in the following table:

Spray scenarios

Scenario	Description	No of applications	Spray interval	Nominal Rate
1	Full (2x)	2	2 weeks	96 g a.s./ha
2	Drift 1 (2x high)	2	2 weeks	Application 1: 36.4 g a.s./ha Application 2: 35 g a.s./ha
3	Drift 2 (2x low)	2	2 weeks	9.1 g a.s./ha
4	Drift 3 (4x low)	4	1 week	4.8 g a.s./ha

Reference treatments were a water control applied during all applications and dimethoate at a rate of 1 L product/ha as reference item, applied during the first and the last application of the fourth test scenario. Application dates and phenological growth stage are presented in the study report in detail.

Application volumes were 500 L/ha, given recommended spray volumes for France, local GAP and crop height and row distance at the site (BBA, 1999).

The trial had a randomized design with 5 replicates (plots of 20 m length, 40 m^2 surface) per treatment. The effects of BYI 08330 OD 150 were expressed in terms of population changes relative to the water control. Population samples were taken shortly before each application, 1 week after the first application and approximately 1, 4 and 8 or 9 weeks after the last application. For test item scenario 4 and water an additional sample was taken 11 weeks after the last treatment. The evaluation was based on time to recovery (=population density similar to control).

Effect values were calculated from total phytoseiid mite numbers according to Abbott (1925) and Henderson-Tilton (1955). In addition, total phytoseiid mite numbers were analysed statistically using the non-parametric Man-Whitney U test.

Findings

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Several acarine taxa were identified. The most abundant taxon prevailing in the vineyard was the predatory mite family Phytoseiidae. Phytoseiid populations almost exclusively consisted of the species *Typhlodromus pyri* (98%-100% throughout the study period). Other mite taxa encountered were Eriophyoidea (rust mites), Tydeidae and Tarsonemidae (fungivorous mites).

	08.06.05	16.06.05	02.07.05	12.07.05	25.07.05	01.08.05	29.08.05
Abbott values							
drift 1 (high 2x)	-1.5%	-11.4%	-5.8%		2.1%		
drift 2 (low 2x)	17.3%	27.6%	14.2%		-1.0%		
drift 3 (low 4x)	13.9%	41.1%		43.8%		31.9%	-17.2%
full (2x)	4.1%	16.3%	36.2%		13.9%		12.8%
reference	18.9%	43.1%		94.1%		95.6%	
Henderson-Tilton values							
drift 1 (high 2x)		-10.1%	-4.4%		3.5%		
drift 2 (low 2x)		12.5%	-3.8%		-18.2%		
drift 3 (low 4x)		31.7%		34.7%		21.0%	-28.7%
full (2x)		12.7%	33.4%		10.3%	94.6%	9.0%
reference		29.8%		92.7%			
P-values (Man-Whitney U test, comparison to water)							
drift 1 (high 2x)	0.834	0.965	0.917		0.917		
drift 2 (low 2x)	0.175	0.076	0.465		0.917		
drift 3 (low 4x)	0.465	***0.028***		0.327		0.017	0.754
full (2x)	0.754	0.347	0.175		0.465		0.754
reference	0.347	***0.009***		***0.014***		***0.009***	

P-values in ***bold italics*** are statistically significant ($P < 0.05$)

BYI 08330 OD 150 had no effect on any of the taxa found, in any of the spray scenarios tested. No statistically significant differences with the water control were found for any of the BYI 08330 OD 150 treatments on all sampling dates, except on 16 June, 5 days after the first treatment, when the 3rd drift rate (4 applications at 4.8 g a.s./ha) showed an effect value of 41% (Abbott) or 31% (Henderson-Tilton). However, on later sampling dates this treatment no longer showed statistically significant effects. Moreover, Abbott effects remained below 50% (Henderson-Tilton effects below 40%) throughout the study. This finding was probably due to natural variation of mite field populations or sampling error, rather than to an actual treatment effect. Population dynamics observed in other test item treatment plots closely resembled fluctuations detected in water control plots. The different spray scenarios of the test item neither induced effects on Eriopheiid mites.

The reference item severely affected Phytoseiid mites in a negative way, with a statistically significant reduction in phytoseiid numbers of 43% to 96%. Thus, the trial is considered valid for the purpose of evaluating potential consequences of test item treatments to predatory mites. Populations of Eriophydoidea could however increase, probably due to reduced predation by phytoseiids.

Conclusion

It is concluded that Spirotetramat OD 150, applied at a nominal rate of 96 g a.s./ha or lower, 2 times in June with a 15-day spray interval, or 4 times at a nominal rate of 4.8 g a.s./ha with a 6 to 8-day spray interval, has no effects on the acarine mite fauna detected with leaf sampling in vineyards in South-West France.

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IIIA1 10.6 Effects on earthworms and other soil macro-organisms

The following overview tables summarise the results of the studies in earthworms and other non-target soil organisms conducted with the active substance spirotetramat and the soil metabolites BYI 08330-cis-ketohydroxy, BYI 08330-enol and 4-methoxy cyclohexanone.

Table IIIA1 10.6-1: Ecotoxicological endpoints for earthworms (spirotetramat and metabolites)

Test organisms	Duration	Test substance	Reference	Ecotoxicological endpoint
Spirotetramat				
Eisenia fetida	acute, 14 d	tech.	KIIA 8.9.1/01	LC_{50} > 1000 mg a.s./kg d.wt.s
BYI 08330-cis-ketohydroxy				
Eisenia fetida	acute, 14 d	metabolite	KIIA 8.9.1/02	LC_{50} > 1000 mg p.m./kg d.wt.s
BYI 08330-methoxy (methoxy-cyclohexanone)				
Eisenia fetida	acute, 14 d	metabolite	KIIA 8.9.1/03	LC_{50} >1000 mg p.m./kg d.wt.s
BYI 08330-enol				
Eisenia fetida	acute, reproduction, 56 d	metabolite	KIIA 8.9.2/01	LC_{50} >1000 mg p.m./kg d.wt.s $NOEC_{Repro}$ 100 mg p.m./kg d.wt.s

Exposure of earthworms

Predicted environmental concentrations in soil (PEC_{soil}) of spirotetramat and its ecotoxicological relevant metabolites in soil, BYI 08330-enol, BYI 08330-ketohydroxy and BYI 08330-methoxycyclohexanone, were calculated by [redacted] (2006, see reports MEF-06/282 and MEF-07/478 for citrus and MEF-06/387 and MEF-08/071 for lettuce) for the use by spray application in citrus and lettuce. The maximum PEC_{soil} values are presented in Table IIIA1 10.6-2.

Table IIIA1 10.6-2: Maximum PECsoil values

Crop	Spirotetramat [mg/kg d.wt.s.]	-enol [mg/kg d.wt.s.]	-ketohydroxy [mg/kg d.wt.s.]	4-methoxy-cyclo-hexanone [mg/kg d.wt.s.]
Citrus	***0.115***	***0.093***	***0.031***	***0.004***
Lettuce	0.029	0.024	0.009	0.003

The values in ***bold italics*** represent the worst case, covering all other application scenarios. These values are used for the Tier I TER calculation.

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IIIA1 10.6.1 Toxicity exposure ratios for earthworms, TER_A and TER_{LT}

Since the acute earthworm test with the active substance spirotetramat was performed in an artificial soil with a reduced organic matter content (5%), the correction factor of 2 for lipophilic substances (log P_{OW} > 2) according to EPPO environmental risk assessment scheme for earthworms has not be applied. No adjustments to correct for the organic matter content as outlined in the Guidance Document for Terrestrial Ecotoxicology (SANCO/10329/2002-final) needs to be considered for spirotetramat metabolites BYI 08330-cis-ketohydroxy and BYI 08330-enol since its log P_{OW} values are <2.

Table IIIA1 10.6.1-1:TER-values for earthworms under worst case assumptions

Test organisms	Test substance	Endpoint [mg a.s/kg d.wt.s.]	PEC_i [mg a.s./kg d.wt.s.]	TER_A	Refined risk assessment required
Eisenia fetida	tech.	LC_{50} > 1000	0.115	**> 8696**	**No**

Even for the maximum PEC_{soil}, the TER_A value clearly meets the Annex VI trigger of 10. Thus, no unacceptable acute risks for earthworms are to be expected by the use of Spirotetramat OD 150 when used as recommended.

Consideration of metabolites – Acute risk assessment

Table IIIA1 10.6.1-2:TER_A for earthworms exposed to metabolites of spirotetramat under worst case assumptions

Test organisms	Test substance	Endpoint [mg p.m./kg d.wt. soil]	PEC_i [mg p.m./kg d.wt.s.]	TER	Refined risk assessment required?
acute					
Eisenia fetida	BYI 08330-enol	LC_{50} > 1000	0.093	**> 10,753**	**No**
Eisenia fetida	BYI 08330-cis-ketohydroxy	LC_{50} > 1000	0.031	**> 32,258**	**No**
Eisenia fetida	BYI 08330-methoxy-cyclohexanone	LC_{50} > 1000	0.004	**> 250,000**	**No**

The Annex VI trigger of 10 is clearly met for the acute exposure of earthworms to BYI 08330-cis-ketohydroxy, BYI 08330-enol and 4-methoxycyclohexanone. Thus, no unacceptable risks for earthworms are to be expected by these soil metabolites of spirotetramat.

Consideration of metabolites – Long-term risk assessment

Due to the extremely short DT_{50} soil of BYI 08330 (0.23 d, see IIIA1 9); DT_{90} 1.1 d, [redacted], 2005, see KIIA 7.2.1/01), a chronic exposure of earthworms to the parent compound can be excluded.
Therefore, it was considered more appropriate to conduct a chronic earthworm study with the enol metabolite rather than with the parent compound. The enol metabolite is the first downstream metabolite of BYI 08330, its soil DT_{50} is 2.95 d (see KIIIA1 9). Soil organisms may thereby be chronically exposed to the enol and further downstream metabolites rather than to the parent compound. For details on the study see AII spirotetramat, KIIA 8.9.2/01.
According to the Terrestrial Guidance Document (SANCO/10329/2002-final), a chronic risk assessment for earthworms is not required since both DT_{90} is < 100 d, and the maximum number of applications per

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year is < 6. Nevertheless, when the endpoint generated in the chronic earthworm study is used for TER calculation, the following TER value is found:

Table IIIA1 10.6.1-3: TER_{LT} for earthworms exposed to metabolites of spirotetramat under worst case assumptions

Test organisms	Test substance	Endpoint [mg/kg d.wt. soil]	PEC_{soil} [mg/kg d.wt.s.]	TER_{LT}	Refined risk assessment required?
Eisenia fetida	BYI 08330-enol	$NOEC_{Repro}$ > 100	0.093	> 1075	No

The Annex VI trigger of 5 is clearly met for the long-term exposure of earthworms to BYI 08330-enol. Thus, no unacceptable risks for earthworms are to be expected by this soil metabolite of spirotetramat.

IIIA1 10.6.2 Acute toxicity to earthworms

No specific acute earthworm toxicity study was conducted with the formulated product Spirotetramat OD 150 since this product contains only one single active ingredient and its toxicity can be predicted based on the data obtained with spirotetramat, where no adverse effects have been found. For results with the active substance see IIA 8.9.

IIIA1 10.6.3 Sublethal effects on earthworms

Based on the triggers stated in the EU-directive 414/EEC and the Terrestrial Guidance Document, a chronic earthworm study for the OD 150 formulation is not required. The DT_{90} in field soils for spirotetramat is very clearly <100 days (1.1 days; worst case value in laboratory test, see KIIA 7.2.1/01, [redacted], 2005) and the maximum number of applications per year is < 6.

IIIA1 10.6.4 Field tests (effects on earthworms)

As no significant acute or sublethal effects have been observed at relevant concentrations (see Table 10.6.1-1) no further studies have to be considered.

IIIA1 10.6.5 Residue content of earthworms

Not required due to the findings presented above.

IIIA1 10.6.6 Effects on other soil non-target macro-organisms

According to the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329/2002-final, tests with additional soil non-target macro-organisms are required only for persistent substances with a field DT_{90} > 100 d. Since the field $DT_{90, soil}$ of spirotetramat is clearly below the trigger value of 100 days (1.1 days; worst case value in laboratory test, see KIIA 7.2.1/01, [redacted], 2005), no concern of effects on other soil non-target macro-organisms due to the use of this compound is indicated and studies on spirotetramat with soil non-target macro-organisms are not required.

<u>Consideration of metabolites</u>

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Even though the $DT_{90, soil}$ of BYI 08330-enol is < 100 days (64.4, see KIIA 7.2.3/01, [redacted], 2006), a study has been conducted with the metabolite and the soil mite *Hypoaspis aculeifer* and the ecotoxicological endpoint is presented in Table IIIA1 10.6.6-1.

Table IIIA1 10.6.6-1: Ecotoxicological endpoints for other soil non-target macro-organisms (BYI 08330-enol)

Test organisms	Duration	Test substance	Reference	Ecotoxicological endpoint
BYI 08330-enol				
Hypoaspis aculeifer	reproduction, 34 d	metabolite	KIIA 8.14/01	$NOEC_{Mortality}$ = 316 mg p.m./kg d.w.t.s

According to the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (SANCO/10329/2002-final, October 2002), a risk assessment is not required.

IIIA1 10.6.7 Effects on organic matter breakdown

Not required due to the findings presented above.

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IIIA1 10.7 Effects on soil microbial activity

Table IIIA1 10.7-1: Summary of effects of spirotetramat on soil micro-organisms

Test system/ Reference	Test substance	Duration	Ecotoxicological endpoint
N-cycle KIIA 8.10.1/01	a.s.	28 d	0.096 kg a.s./ha and 0.96 kg a.s./ha: No influence on nitrogen transformation
C-cycle KIIA 8.10.2/01	a.s.	28 d	0.096 kg a.s./ha and 0.96 kg a.s./ha: No influence on carbon transformation

Remark
In the soil microbial studies referred to above, the highest rate tested was the ten-fold overdose of the application rate for a canopy height of 1 m. However, the rates tested nevertheless cover as well the highest total hectare application rate for citrus of 288 g a.s./ha at 3 m CH, since in a realistic application scenario a crop interception of 70% has to be considered for all growth stages of citrus, as outlined in FOCUS Groundwater (see [redacted], 2006; Report No. MEF-06/282).

Risk assessment
The results presented show that during the 28-day test, the one-fold rate of spirotetramat (96 g a.s./ha, corresponding to 0.128 mg a.s./kg d.wt.s.) and the 10-fold overdose (960 g a.s./ha, corresponding to 1.28 mg a.s./kg d.wt.s.) of the compound do not negatively influence the metabolic activity of the microbial biomass.

Thus, no unacceptable risks to soil non-target micro-organisms are to be expected from the use of Spirotetramat OD 150.

IIIA1 10.7.1 Laboratory test to investigate impact on soil microbial activity

For results with the active substance please refer to AII 8.10.1 and AII 8.10.2.

IIIA1 10.7.2 Further testing to investigate impact on soil microbial activity

Not necessary due to the findings presented above.

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IIIA1 10.8 Effects on non-target plants

IIIA1 10.8.1 Effects on non-target terrestrial plants

The risk assessment for non-target terrestrial plants is based on the “Guidance Document on Terrestrial Ecotoxicology”, SANCO/10329/2002 rev2 final, 17 October 2002.
In the case of a non-herbicide, screening results and/or Tier 1 studies give first information about the likelihood for terrestrial plant effects. The risk can be considered acceptable if there are no data indicating more than 50% phytotoxic effect at the maximum application rate. Where a 50% effect is identified in one or more species in the tier 1 studies, tier 2 dose response studies are triggered to identify the ER_{50} values of these species and these endpoints are used to determine if mitigation (in-crop buffers and/or drift reduction technology) is necessary. Such mitigation can be refined by the use of higher tier field or semi-field studies.

Table IIIA1 10.8-1: Summary of effects on non-target terrestrial plants

Terrestrial Non-Target Plants			
Number of species tested	**Test method** **Test substance** **Application rate**	**Effects**	**Reference**
Dicotyledoneae: 4 (oilseed rape, sunflower, cucumber, soybean) Monocotyledoneae: 2 (oats, corn)	Post-emergence (vegetative vigour) test Spirotetramat OD 150 288 g a.s./ha	Effects on biomass were oilseed rape (83.3%); sunflower (43.1%); cucumber (41.3%); soybean (40.2%); oats (56.6%) and corn (56.9%)	KIIA 8.12/01
Dicotyledoneae: 6 (cucumber, canola, soybean, sunflower, sugarbeet, tomato) Monocotyledoneae: 4 (corn, oat, ryegrass, onion)	Vegetative vigour Spirotetramat OD 150 Monocot: 0, 11, 22, 44, 88 and 176 g a.s./ha Dicots: 0 and 176 g a.s./ha	ER_{50} dry weight (biomass): 134 g a.s./ha (corn) ER_{50} plant survival: >176 g a.s./ha (corn) ER_{50} plant length: 172 g a.s./ha (corn)	KIIA 8.12/02
Dicotyledoneae: 1 (oilseed rape) Monocotyledoneae: 2 (oat, corn)	Vegetative vigour Spirotetramat OD 150 25.5, 72, 144 and 288 g a.s./ha	ER_{50} > 288 g a.s./ha (oilseed rape, oat and corn)	KIIA 8.12/03
Dicotyledoneae: 4 (oilseed rape, sunflower, cucumber, soybean) Monocotyledoneae: 2 (oats, corn)	Pre-emergence (seedling emergence) test: Spirotetramat OD 150 288 g a.s./ha	None of the tested plant species showed pronounced phytotoxic effects at the application rate of 3 kg product/ha; all visual or measured effects were <50% trigger for further testing	KIIA 8.12/04
Dicotyledoneae: 6 (cucumber, canola, soybean, sunflower, sugarbeet, tomato) Monocotyledoneae: 4 (corn, oat, ryegrass, onion)	Seedling emergence and growth Spirotetramat OD 150 176 g a.s./ha	No significant adverse effects > 25%	KIIA 8.12/05
Monocotyledoneae: 3 (corn, oat, ryegrass)	Higher tier vegetative vigour Spirotetramat OD 150 18, 36, 72, 144 & 288 g a.s./ha with assessments	ER_{50} dry weight (biomass) values for corn 152.2 & 149.2 g a.s./ha; values for oat and ryegrass >288 g a.s./ha	KIIIA1 10.8.1.4/01

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	at 21 and 35-36 days		

In Tier 1 seedling emergence and growth studies with the formulation, there were no pronounced effects on non-target terrestrial plants at application rates up to 288 g a.s./ha (KIIA 8.12/04, KIIA 8.12/05). All effects were <50% and hence below the 50% trigger for further testing.
However, in the Tier 1 vegetative vigour test (KIIA 8.12/01) effects > 50% were found in three of the species tested and thus, a vegetative vigour tier 2 test was triggered for the affected species. In this Tier 2 test (KIIA 8.12/03) the ER_{50} for all species included (oilseed rape, oat and corn) was > 288 g a.s./ha. In a further vegetative vigour Tier 1 and 2 study performed with Spirotetramat OD 150 (KIIA 8.12/02) the lowest ER_{50} of 134 g a.s./ha was determined for corn, the most sensitive of the species tested.

Exposure assessment
Effects on non-target plants are of concern in the off-field environment, where they may be exposed to spray drift. For two applications to citrus, 12.13% and 6.81% of the full application rate of 288 g a.s./ha are assumed to reach areas at 3 m and 5 m from the edge of the crop, respectively. For two applications to lettuce, 2.38% and 0.47% of the full application rate of 72 g a.s./ha are assumed to reach areas at 1 m and 5 m from the edge of the crop, respectively. The amount of spray drift from two applications reaching off-crop habitats is calculated using the 82nd percentile estimates derived by the BBA (2000)[15] from spray-drift predictions of Ganzelmeier & Rautmann (2000)[16].
Moreover, multiple application factors (MAF) based on the minimum spray intervals have to be considered. The MAF for the use in citrus (2 applications, 21 days interval) and lettuce (2 applications, 14 days interval) were calculated to be 1.23 and 1.4, respectively. The MAF were determined by using the equation for the calculation of average residue levels (MAF_m) as provided in Appendix H of the Guidance Document for Risk Assessment on Birds and Mammals[17]. The corresponding off-field predicted environmental rates ($PER_{off\text{-}field}$) are presented in the table below.

Table IIIA1 10.8-2: Predicted environmental rates (PER) at different distances from the field edge

Crop	Timing of application	Number of applications	Maximum single rate [g a.s./ha]	MAF	PER [g a.s./ha] at distance		
					1m	3m	5m
Citrus	BBCH 71-78	2	288	1.23	n.a.	42.97	24.12
Lettuce	BBCH 42-43	2	72	1.4	2.40	n.a.	0.47

n.a.: not applicable

Deterministic risk assessment
The following deterministic risk assessment is based on the findings of the vegetative vigour Tier 1 and Tier 2 study (KIIA 8.12/02) which, among all non-target plant tests performed with Spirotetramat OD 150, delivered the lowest ER50 of 134 g a.s./ha (corn, shoot dry weight).

According to the Terrestrial Guidance Document, the risk to non-target terrestrial plants is assessed by comparing the exposure in field margins caused by drift with the lowest ER_{50} obtained from the non-target plant studies. An assessment factor of 5 is required in order to prove safe use.

[15] BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.
[16] Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.
[17] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. [139 pp.].

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Table IIIA1 10.8-3: Deterministic TER calculation for the use of the product in citrus (= fruit trees late) based on a maximum use rate of 288 g a.s/ha with and without 50% drift reducing spray equipment using the 134 g a.s./ha endpoint for corn from the tier 2 glasshouse study

distance from field	% Drift		PER in g a.s./ha		TERs	
	Conventional spray equipment	50% drift reduction	Conventional spray equipment	50% drift reduction	Conventional spray equipment	50% drift reduction
3 m	12.13	6.07	42.97	21.49	**3.1**	6.2
5 m	6.81	3.41	24.12	12.06	5.6	11.1

Bold letters: TERs which do not meet the trigger of 5

Table IIIA1 10.8-4: Deterministic TER calculation for the use of the product in lettuce based on a maximum use rate of 72 g a.s./ha with and without 50% drift reducing spray equipment using the 134 g a.s./ha endpoint for corn from the tier 2 glasshouse study

distance from field	% Drift		PER in g a.s./ha		TERs	
	Conventional spray equipment	50% drift reduction	Conventional spray equipment	50% drift reduction	Conventional spray equipment	50% drift reduction
1 m	2.38	1.19	2.40	1.20	55.8	112
5 m	0.47	0.24	0.47	0.24	285	558

Based on the lowest ER_{50} endpoint determined for non-target terrestrial plants, a TER of >5 can be achieved for citrus by using 50% drift reducing equipment or a 5 m in-crop buffer. To refine this mitigation a higher tier study has been conducted (see IIIA1 10.8.4/01) and the findings from this will be used in the higher tier risk assessment. For use in lettuce the TER is well in excess of 5 already at 1 m distance and the risk to non-target terrestrial plants can be considered acceptable without any mitigation measures.

Refined risk assessment

In the higher tier vegetative vigour semi-field study (IIIA1 10.8.4/01) in which the three most sensitive species from the glasshouse studies were tested, ER_{50} values for biomass of oat and ryegrass exceeded the maximum use rate of 288 g a.i./ha. Corn was again the most sensitive species with ER_{50} values for biomass of 152.2 and 149.2 g a.s./ha from the first and second harvest periods, respectively. These values indicate that corn is less sensitive under more relevant environmental conditions. The refined deterministic TER calculation based on the lowest endpoint of 149.2 g a.s./ha is shown in the table below.

According to the Terrestrial Guidance Document the trigger of 5 may be reduced if information on more than 6 species is available. In total, 10 different species have been tested in vegetative vigour studies with the formulation. Moreover, the endpoint for the refined risk assessment derives from a higher tier study and effects of the product are only on the sub-lethal endpoint biomass, and not on lethality (i.e. there is no effects on survival even at the maximum rate of 288 g a.i./ha). For these reasons, it is considered justified to lower the trigger from 5 to 2.

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Table IIIA1 10.8-5: Refined deterministic TER calculation for the use of the product in citrus (= fruit trees late) based on a maximum use rate of 288 g a.s/ha with and without 50% drift reducing spray equipment using the 149.2 g a.s./ha endpoint for corn from the higher tier semi-field study

distance from field	% Drift		PER in g a.s./ha		TERs	
	Conventional spray equipment	50% drift reduction	Conventional spray equipment	50% drift reduction	Conventional spray equipment	50% drift reduction
3 m	12.13	6.07	42.97	21.49	3.5	6.9
5 m	6.81	3.41	24.12	12.06	6.2	12.4

Based on the ER_{50} endpoint determined in the higher tier study for non-target terrestrial plants, the refined TER of >2 is already achieved with conventional spray equipment at 3 m distance.

Conclusion
It is concluded that the use of the product in citrus (2x 288 g a.s./ha) and lettuce (2x 72 g a.s./ha) will not produce unacceptable effects on terrestrial non-target plants growing near treated fields. No mitigation measures are required.

IIIA1 10.8.1.1Seed germination

The endpoint "seed germination" is addressed in the studies on seedling emergence (see IIIA1 10.8.1.3)

IIIA1 10.8.1.2Vegetative vigour

See KIIA 8.12/01, KIIA 8.12/02 and KIIA 8.12/03

IIIA1 10.8.1.3Seedling emergence

See KIIA 8.12/04 and KIIA 8.12/05

IIIA1 10.8.1.4Terrestrial field testing

A higher tier semi-field study has been conducted with the three most sensitive species and this is summarised below:

Report: **KIIIA1 10.8.1.4/01, [redacted]; 2008**
Title: The phytotoxic effects of Spirotetramat OD 150B G on the vegetative vigour of three plant species determined under semi-field conditions.
Date: 2008-09-22
Organisation: Bayer CropScience GmbH, [redacted], Germany
Report No.: HT 08/008; M-307459-01-1
Publication: unpublished
Dates of experimental work: June 18, 2008 -July 29, 2008
Guidelines: OECD Guideline for the Testing of Chemicals, Guideline 227: Terrestrial Plant test: Vegetative Vigour Test, July 2006 adapted for a higher tier study.

Deviations:	Guideline adapted to the purpose of this higher tier study (plants kept under semi-field conditions; the duration of exposure was extended to allow a longer growth interval after spray application of the product).
GLP:	yes (certified laboratory)

Executive summary

The objective of this specific study was to evaluate the effect of Spirotetramat OD 150B G (102.1% of nominal) on the vegetative vigour of three plant species representing one monocotyledonous plant family under external conditions. These monocotyledonous species were identified as being the most sensitive in tier 1 and 2 NTTP studies.

The design of this higher tier non-target terrestrial plant study is based on OECD 227 in which the primarily aim is to generate ER_{50} values, with differences in the duration of exposure, to allow an assessment of plant recovery from the adverse effect of the test item.

In total, plants of three monocotyledonous species were tested under semi-field conditions: oat (*Avena sativa*), ryegrass (*Lolium perenne*) and corn (*Zea mays*).

At the 2-4 leaf stage serial dilutions of Spirotetramat OD 150B G were sprayed with application rates ranging from 288 g a.i./ha down to 18 g a.i./ha. Control plants were sprayed with deionised water.

The parameters measured were: visual phytotoxicity, mortality, shoot length and plant biomass (shoot dry weight).

Endpoint assessments were conducted at two harvest dates: the first endpoint assessment was 3 weeks after application (at day 21 after application for phytotoxicity, survival, growth stage, shoot length and shoot dry weight) and the second endpoint assessment was 5 weeks after application (at day 35 after application for phytotoxicity, survival, growth stage and shoot length, and at day 36 shoot dry weight).

Statistical analysis of data was performed to obtain NOER, LOER, ER_{25} and ER_{50} values for survival, shoot length and biomass (shoot dry weight).

The most sensitive monocotyledonous species in this higher tier study was corn. The lowest ER25 values were for shoot dry weight with 75.8g a.i./ha at the 1st harvest and 80.9g a.i./ha at the 2nd harvest. The lowest **ER_{50}** values were also for shoot dry weight with **152.2g a.i./ha** at the 1st harvest and **149.2g a.i./ha** at the 2nd harvest. The NOER values for both shoot length and dry weight were <18g a.i./ha at the 1st harvest, however, these were 72g a.i./ha at the 2nd harvest, indicating recovery.

MATERIAL AND METHODS

A Materials

1. Test material	Spirotetramat OD 150B G (BYI 08330; AE 1302943)
Description	Light, brown suspension
Batch No.	2007-005113
Material No.:	06082040
Specification No.:	102000016434
Density (20 °C)	0.989 g/mL
Content a.i.	152 g/L (analysed)
Expiration date	2010-04-22
Testing rates	18, 36, 72, 144, 288 g a.i./ha
2. Vehicle and/or positive control	deionized water
3. Test plants	
Species	*Avena sativa, Lolium perenne, Zea mays*
Source	Seeds were supplied from commercial sources via Bayer CropScience AG, Horticulture, H872, [redacted].
Storage seeds	Seeds were stored in plastic boxes in the refrigerator.
Storage plants	Plants were grown in pots and held under semi-field conditions in an outdoor cage that protected the

	plants and pots from damage due to heavy rainfall and to a loss of compound from the test system
Age of plants	Pots were sown with more than 4 seeds and thinned to 4 plants per pot prior application of the test item. Plants were grown in pots prior testing to reach the 2-4 leaf stage for application.
4. Test soil	
Soil type	Standard soil (silt loam) The soil was sieved to 2 mm. The soil was analysed separately with GLP in the laboratory of [redacted]
Source	Bayer CropScience AG, Global Biology Herbicides, Horticulture, H 872, [redacted]
Sterilisation	120 degrees vapour for about 30 minutes.
Fertilization	2.4 g Blaukorn per liter
Composition and particle size	0.05 - 2.0 mm sand 6.7 % < 0.002 mm clay 24.2 % 0.002 - 0.05 mm silt 59.1 %
Cation capacity	17.0 meq /100 g
Lime content	1.2 % $CaCO_3$
Organic carbon content	1.30 % C
pH - value	7.31

B Study design and methods

1. In life dates	June 18, 2008 – July 29, 2008
Test duration	42 d
2. Experimental treatments	
Test set up	Plants were grown in commercial plastic pots: Pot size: 21 cm (*Avena sativa, Zea mays*) 13 cm (*Lolium perenne*) **Experimental design for each plant species:** No. plants per pot: 4 No. pots per treatment group: 16 No. pots per harvest time: 8
Watering:	After application of the test item, irrigation was achieved by bottom watering via saucers standing below each pot. Water was given and retained within the saucer according to the need of the plants in order to have an optimal water supply for plant growth.
Fertilizers, and crop protection agents:	1 mL Universaldünger from Bayer-Garten was given 16 days after application for ryegrass and 2 mL for oat and corn. On day 26, oat plants (for 2nd harvest) were sprayed with Input EC460 against fungal infection (0.5% v/v).

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Exposure Time:	The post emergent plants were sprayed on June 18th 2008 Two exposure times were used: 3 weeks after application with the first harvest on Day 21; and 5 weeks after application with a second harvest on Day 36.
Climatic conditions	
Test Environment:	Outdoor area enclosed within a cage, adjacent to the glasshouse area at Bayer CropScience AG, H874, [redacted]. Plants were moved indoors for application of the test item by the laboratory sprayer, then returned to the cage.
Environmental conditions:	Temperature and humidity were recorded with thermohydrograph throughout the study. Additionally, climatic conditions were recorded from the Global Biology Herbicide and included to the raw data (Data not under GLP, only for information).
Light intensity:	Light intensity was recorded from the Global Biology Herbicide (Data not under GLP, only for information).
3. Observations	
Phytotoxicity Records (chlorosis, necrosis, bleaching, wilting, leaf deformation, stunting):	Visual phytotoxicity ratings of living plants at days 7, 14, 21, 28 and 35 according to EPPO Standard 135 from surviving plants.
Survival:	Number of plants that survived after application were recorded at days 7, 14, 21, 28 and 35.
Shoot length:	Shoot length was determined from individual surviving plants at the final assessments (day 21 for the 1st harvest and day 35 for the 2nd harvest).
Growth Stages:	Growth stages at the final assessments were reported according to BBCH-Monograph - Growth stages (day 21 for the 1st harvest and day 35 for the 2nd harvest).
Plant biomass:	Shoot dry weight was determined at the final assessments (day 21 for the 1st harvest and day 36 for the 2nd harvest). The surviving plants of one pot represent one replicate.

Procedure: The aim of the study was the examination of the phytotoxicity of Spirotetramat OD 150B G on three monocotyledonous species: oat (*Avena sativa*), ryegrass (*Lolium perenne*) and corn (*Zea mays*).

The plants were grown from seeds in pots in a cage covered with a clear Plexiglas roof, which protected the pots from damage due to heavy rainfall and to a loss of compound from the test system. Seeds used on the study had not been treated with pesticides or repellents prior to test initiation. The test item was dissolved in deionized water and was applied once with 100 L/ha using an spray chamber equipped with an overhead nozzle (Tracksprayer SprayLab SLGH 2500, Teejet 8001 EVS), with nozzle height set at 30 cm above the sprayed surface. Speed: 3.0 km/h; Pressure: 2.0 bar. The blank control spray solution was 100 L/ha deionized water. The spray chamber volume was calibrated by weighting the amount of water applied to a known

surface area The spray chamber and nozzle system simulate normal field application of the product. The pots were placed indiscriminately for each species.
The parameters measured were: visual phytotoxicity, mortality, shoot length and plant biomass (shoot dry weigth). Observations for phytotoxicity and survival were made at 7 day intervals (days 7, 14, 21, 28 and 35). Growth stages and shoot length were taken at day 21 and 35. The biomass (shoot dry weight) endpoint assessment was made at two time points after the post emergent spray application: one harvest was at 3 weeks (Day 21) and the final harvest at approximately 5 weeks (Day 36).

4. Statistics

Survival	Number of plants that survived after application in comparison to the control at the end of the each assessment period.
Phytotoxicity	Individual phytotoxicity for replicates were expressed as means in summary tables.
Shoot length	The mean shoot length for each replicate was compared to those of the untreated controls for both final assessments.
Biomass	The mean dry weight for each replicate were compared to those of the controls for both final assessments.
Statistical Analysis	Survival, shoot length and shoot biomass (shoot dry weight) were compared using the ToxRat software for statistical analysis (version 2.09).
Detrimental Effect Levels	ER_{25} and ER_{50} with the 95 percent confidence limits as well as the LOER (Lowest Observed Effect Rate) and NOER (No Observed Effect Rate). If the NOER is calculated as greater than the highest rate tested, it will be reported as the highest rate tested (without > or >=) excepted in the ToxRat calculations.

RESULTS AND DISCUSSION

A. Findings

Analysis of Spirotetramat OD 150B G of the highest application rate revealed it to be 102.1% of nominal.
The Day 21 (1st harvest) and day 35-36 (2nd harvest) No Observed Effect Rate (NOER), Lowest Observed Effect Rate (LOER), and ER_{25} and ER_{50} values expressed as g a.i./ha are summarised for each of the plant species in the following tables:

For clarity reason in the following tables, the results are expressed in "1st and 2nd harvest" and not in days.

Higher Tier Plant Survival								
Plant Species	ER_{25} (g a.i./ha)	95% Confidence Limits		ER_{50} (g a.i./ha)	95% Confidence Limits		LOER (g a.i./ha)	NOER (g a.i./ha)
		lower	upper		lower	upper		
Oat 1st harvest	>288#	-	-	>288#	-	-	>288#	288#
Oat 2nd harvest	>288#	-	-	>288#	-	-	>288#	288#
Ryegrass 1st harvest	>288#	-	-	>288#	-	-	>288#	288#
Ryegrass 2nd harvest	>288#	-	-	>288#	-	-	>288	288
Corn 1st harvest	>288#	-	-	>288#	-	-	>288#	288#
Corn 2nd harvest	>288#	-	-	>288#	-	-	>288#	288#

-: confidence limits not determined or >highest test concentration
#: extrapolated values, calculated values were outside the range tested or not determined

Higher Tier Plant Shoot Length								
Plant Species	ER_{25} (g a.i./ha)	95% Confidence Limits		ER_{50} (g a.i./ha)	95% Confidence Limits		LOER (g a.i./ha)	NOER (g a.i./ha)
		lower	upper		lower	upper		
Oat 1st harvest	>288#	-	-	>288#	-	-	288	144
Oat 2nd harvest	>288#	-	-	>288#	-	-	288	144
Ryegrass 1st harvest	>288#	-	-	>288#	-	-	>288	288
Ryegrass 2nd harvest	>288#	-	-	>288#	-	-	>288	288
Corn 1st harvest	110.2	59.8	147.7	228.9	172.9	-	<18	<18
Corn 2nd harvest	185.3	36.9	257.8	>288#	238.9	-	144	72

-: confidence limits not determined or >highest test concentration
#: extrapolated values, calculated values were outside the range tested or not determined

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Higher Tier Plant Biomass (shoot dry weight)								
Plant Species	ER_{25} (g a.i./ha)	95% Confidence Limits		ER_{50} (g a.i./ha)	95% Confidence Limits		LOER (g a.i./ha)	NOER (g a.i./ha)
		lower	upper		lower	upper		
Oat 1st harvest	206.6	37.6	-	>288#	280.0	-	144	72
Oat 2nd harvest	>288#	-	-	>288#	-	-	>288	288
Ryegrass 1st harvest	>288#	-	-	>288#	-	-	>288	288
Ryegrass 2nd harvest	>288#	-	-	>288#	-	-	>288	288
Corn 1st harvest	75.8	19.4	115.4	152.2	95.1	-	<18	<18
Corn 2nd harvest	80.9	17.8	121.5	149.2	90.5	283.1	144	72

-: confidence limits not determined or >highest test concentration
#: extrapolated values, calculated values were outside the range tested or not determined

Comments on the different plant species tested

Oat (*Avena sativa*) 1st harvest
The foliar treatment of Spirotetramat OD 150B G applied at five application rates of 18, 36, 72, 144 and 288g a.i./ha resulted in no significant impact on the **survival** of oat plants at any application rate tested. The NOER for this endpoint was set at 288g a.i./ha and the ER_{25} and ER_{50} values were set for both as >288g a.i./ha.
There were significant effects on **shoot length** at the highest application rate of 288g a.i./ha. The NOER for this endpoint was 144g a.i./ha. The ER_{25} and ER_{50} values for shoot length were set for both as >288g a.i./ha.
Shoot dry weight (biomass) was significantly reduced at application rates including and above 144g a.i./ha. The NOER with respect to biomass was 72g a.i./ha. The ER_{25} value for biomass was calculated as 206.6g a.i./ha. The ER_{50} value was set as >288g a.i./ha.
Oat (*Avena sativa*) 2nd harvest
The foliar treatment of Spirotetramat OD 150B G resulted in no significant impact on the **survival** of oat plants at any application rate tested. The NOER for this endpoint was set at 288g a.i./ha and the ER_{25} and ER_{50} values were set for both as >288g a.i./ha.
There were significant effects on **shoot length** at the highest application rate of 288g a.i./ha. The NOER for this endpoint was 144g a.i./ha. The ER_{25} and ER_{50} values for shoot length were set for both as >288g a.i./ha.
Shoot dry weight (biomass) was not significantly reduced at any application rate tested. The NOER with respect to biomass was the highest rate tested of 288g a.i./ha. The ER_{25} and ER_{50} values for shoot dry weight were set for both as >288g a.i./ha.

Ryegrass (*Lolium perenne*) 1st harvest
The foliar treatment of Spirotetramat OD 150B G applied at five application rates of 18, 36, 72, 144 and 288g a.i./ha resulted in no significant impact on the **survival** of ryegrass plants at any application rate tested. The NOER for this endpoint was set at 288g a.i./ha and the ER_{25} and ER_{50} values were set for both as >288g a.i./ha.
Shoot length was not significantly reduced at any application rate tested. The NOER for this endpoint was the highest rate tested of 288g a.i./ha. The ER_{25} and ER_{50} values for shoot dry weight were set for both as >288g a.i./ha.
Shoot dry weight (biomass) was not significantly reduced at any application rate tested. The NOER

with respect to biomass was the highest rate tested of 288g a.i./ha. The ER_{25} and ER_{50} values for shoot dry weight were set for both as >288g a.i./ha.

Ryegrass (*Lolium perenne*) 2nd harvest

The foliar treatment of Spirotetramat OD 150B G resulted in no significant impact on the **survival** of ryegrass plants at any application rate tested. The NOER for this endpoint was calculated as 288g a.i./ha and the ER_{25} and ER_{50} values were set for both as >288g a.i./ha.

Shoot length was not significantly reduced at any application rate tested. The NOER for this endpoint was the highest rate tested of 288g a.i./ha. The ER_{25} and ER_{50} values for shoot dry weight were set for both as >288g a.i./ha.

Shoot dry weight (biomass) was not significantly reduced at any application rate. The NOER with respect to biomass was the highest rate tested of 288g a.i./ha. The ER_{25} and ER_{50} values for shoot dry weight were set for both as >288g a.i./ha.

Corn (*Zea mays*) 1st harvest

The foliar treatment of Spirotetramat OD 150B G applied at five application rates of 18, 36, 72, 144 and 288g a.i./ha resulted in no significant impact on the **survival** of corn plants at any application rate tested. The NOER for this endpoint was set at 288g a.i./ha and the ER_{25} and ER_{50} values were set for both as >288g a.i./ha.

There were significant effects on **shoot length** at all application rates tested. The NOER for this endpoint was calculated as <18g a.i./ha. The ER_{25} was calculated as 110.2g a.i./ha. The ER_{50} value was calculated as 228.9g a.i./ha

Shoot dry weight (biomass) was significantly reduced at all application rates tested excepted at the application rate of 36g a.i./ha. The NOER with respect to biomass was calculated as <18g a.i./ha. The ER_{25} value for biomass was calculated as 75.8g a.i./ha. The ER_{50} value was calculated as 152.2g a.i./ha.

Corn (*Zea mays*) 2nd harvest

The foliar treatment of Spirotetramat OD 150B G applied at five application rates of 18, 36, 72, 144 and 288g a.i./ha resulted in no significant impact on the **survival** of corn plants at any application rate tested. The NOER for this endpoint was set at 288g a.i./ha and the ER_{25} and ER_{50} values were set for both as >288g a.i./ha.

There were significant effects on **shoot length** at application rates including and above 144g a.i./ha. The NOER for this endpoint was calculated as 72g a.i./ha. The ER_{25} was calculated as 185.3g a.i./ha. The ER_{50} value was set as >288g a.i./ha.

Shoot dry weight (biomass) was significantly reduced at application rates including and above 144g a.i./ha. The NOER with respect to biomass was calculated as 72g a.i./ha. The ER_{25} value for biomass was calculated as 80.9 g a.i./ha. The ER_{50} value was calculated as 149.2g a.i./ha.

B. Observations

Typical symptoms with Spirotetramat OD 150B G observed in this study were chlorosis, necrosis, leaf deformation, wilting, stunting and lodging. The presence and severity of these symptoms differed with application rates and species sensitivity to the product.

Phytotoxicity effects of Spirotetramat OD 150B G

Data of phytotoxicity effects for oat, rye grass and corn are summarised in the tables below.

Explanation of phytotoxicity codes:

0: no injury or effect
A: slight symptom(s)
B: moderate symptom(s)
C: severe symptom(s)

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D: total plant symptom(s)
E: moribund
a = chlorosis (yellowing of green shoot tissue);
b = necrosis (brown shoot tissue)
c = bleaching (shoot tissue without any pigmentation)
d = wilting (loss of turgor of shoot tissue)
e = leaf deformation (leaf curl, abnormal leaf shape)
f = stunting (plant height reduced with shorter inter-node lengths)
g =lodging (plants fallen down)

BBCH data represents the mean value of the growth stages of the replicates for each dose according to the phenological growth stages and BBCH identification keys of weed species from the Compendium of Growth Stage Identification Keys for Mono- and Dicotylenous plants (2nd edition, 1997).

Phytotoxicity Oat

	Phytotoxicity Oat 1st harvest			BBCH
g a.i./ha	**Day 7**	**Day 14**	**Day 21**	**Day 21**
control	0	0	0	31
18	0	0	0	31
36	0	0	0	31
72	0-A*d*	0-A*e*	0-A*f*	31
144	A*df*	0-A*f*	0-A*f*	31
288	A-B*adf*	A-B*ef*	0-A*f*	31

	Phytotoxicity Oat 2nd harvest					BBCH
g a.i./ha	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 35**
control	0	0	0	0	0	51-55
18	0	0	0	0	0	51-55
36	0	0	0	0	0	51-55
72	0-A*d*	0	0	0	0	51-55
144	0-A*df*	0-A*ef*	0	0	0	51-55
288	A-B*adf*	0-B*ef*	0-A*f*	0	0-A*f*	51-55

Phytotoxicity Ryegrass

	Phytotoxicity Ryegrass 1st harvest			BBCH
g a.i./ha	Day 7	Day 14	Day 21	Day 21
control	0	0	0	23-25
18	0	0	0	23-25
36	0	0	0	23-25
72	0	0	0	23-25
144	0-B*adf*	0	0	23-25
288	A-B*adf*	0-A*f*	0-A*f*	23-25

	Phytotoxicity Ryegrass 2nd harvest					BBCH
g a.i./ha	Day 7	Day 14	Day 21	Day 28	Day 35	Day 35
control	0	0	0	0	0	25-29
18	0	0	0	0	0	25-29
36	0	0	0	0	0	25-29
72	0	0	0	0	0	25-29
144	0-A*adf*	0	0	0	0	25-29
288	A-C*adf*	0-A*df*	0-A*f*	0	0	25-29

Phytotoxicity Corn

	Phytotoxicity Corn 1st harvest			BBCH
g a.i./ha	Day 7	Day 14	Day 21	Day 21
control	0	0	0	31
18	0	0	0	31
36	0-A*a*	0-A*a*	0	31
72	A-C*ad*	0-B*abf*	A*abf*	31
144	C*ad*	B-C*abef*	B-C*abfg*	31
288	C-D*adf*	D*abef*	C-D*abefg*	12-31

	Phytotoxicity Corn 2nd harvest					BBCH
g a.i./ha	Day 7	Day 14	Day 21	Day 28	Day 35	Day 35
control	0	0	0	0	0	33
18	0	0	0	0	0-A*f*	33
36	0-A*a*	0-A*a*	0	0-A*f*	0-A*f*	33
72	A-B*ad*	A*af*	A*af*	A-B*abf*	A*a*	33
144	C-D*adf*	C*abef*	C*abfg*	B-C*abfg*	A-B*af*	32-33
288	C-D*adf*	C-D*abef*	C-D*abfg*	C-D*abfg*	C-D*abfg*	31-33

Data represents the mean value for the phytotoxicity ratings of the replicates for each dose.

Comments for each plant species

Observations for phytotoxicity and survival were made at 7 day intervals (days 7, 14 and 21 for the 1st harvest; days 7, 14, 21, 28 and 35 for the 2nd harvest).

For reasons of clarity, the results are expressed in "1st and 2nd harvest" and not in days.

Oat (*Avena sativa*) 1st harvest

Phytotoxic symptoms observed in oat plants during the study (1st harvest) included chlorosis, wilting, leaf deformation and stunting.

Marginal phytotoxic symptoms occurred at test end as stunting were observed at the application rates including and above 72g a.i./ha.

There was no effect on growth stage development of treated oat plants in comparison to the untreated controls at any application rate tested.

Oat (*Avena sativa*) 2nd harvest

Phytotoxic symptoms observed in oat plants during the study (2nd harvest) included chlorosis, wilting, leaf deformation and stunting.

Marginal phytotoxic symptoms occurred at test end as stunting were observed at the highest application rate tested of 288g a.i./ha.

There was no effect on growth stage development of treated oat plants in comparison to the untreated controls at any application rate tested.

Ryegrass (*Lolium perenne*) 1st harvest

Phytotoxic symptoms observed in ryegrass plants during the study (1st harvest) included chlorosis, wilting and stunting.

Marginal phytotoxic symptoms occurred at test end as stunting were observed at the highest application rate tested of 288g a.i./ha.

There was no effect on growth stage development of treated ryegrass plants in comparison to the untreated controls at any application rate tested.

Ryegrass (*Lolium perenne*) 2nd harvest

Phytotoxic symptoms observed in ryegrass plants during the study (2nd harvest) included chlorosis, wilting and stunting.

No phytotoxic symptom was observed at test end at any application rate tested

There was no effect on growth stage development of treated ryegrass plants in comparison to the untreated controls at any application rate tested.

Corn (*Zea mays*) 1st harvest

Phytotoxic symptoms observed in corn plants during the study (1st harvest) included chlorosis, necrosis, wilting, leaf deformation, stunting and lodging.

Slight phytotoxic symptoms were visible at test end as chlorosis, necrosis and stunting were observed at the application rate of 72g a.i./ha.

Moderate to severe phytotoxic symptoms were visible at test end as chlorosis, necrosis, leaf deformation, stunting and lodging at application rates including and above 144g a.i./ha.

There were effects on growth stage development of treated plants in comparison to the untreated controls at the highest application rate tested of 288g a.i./ha.

Corn (*Zea mays*) 2nd harvest

Phytotoxic symptoms observed in corn plants during the study (2nd harvest) included chlorosis, necrosis, wilting, leaf deformation, stunting and lodging.

Slight phytotoxic symptoms were visible at test end as chlorosis, necrosis and stunting were observed at the application rate including and above 18g a.i./ha.

Severe phytotoxic symptoms were visible at test end as chlorosis, necrosis, stunting and lodging at at the highest application rate tested of 288g a.i./ha.

There were effects on growth stage development of treated plants in comparison to the

untreated controls at the two highest application rates tested of 144 and 288g a.i./ha.

CONCLUSION

The most sensitive monocotyledonous species to Spirotetramat OD 150B G in this higher tier study in which plants were grown and maintained under external environmental conditions was corn.
The lowest ER_{25} values were for shoot dry weight with 75.8g a.i./ha at the 1st harvest and 80.9g a.i./ha at the 2nd harvest.
The lowest ER_{50} values were also for shoot dry weight with 152.2g a.i./ha at the 1st harvest and 149.2g a.i./ha at the 2nd harvest.
The NOER values for both shoot length and dry weight were <18g a.i./ha at the 1st harvest, however, these were 72g a.i./ha at the 2nd harvest, indicating recovery.

IIIA1 10.8.2 Effects on non-target aquatic plants

Table IIIA1 10.8.2-1: Ecotoxicological endpoints for non-target aquatic plants (spirotetramat and metabolite)

Test organisms	Test system	Test substance	Reference	Ecotoxicological endpoint
Spirotetramat				
Lemna gibba	7 d, static-renewal	a.s.	KIIA 8.6/01	E_rC_{50} 6.21 mg a.s./L[1]
BYI 08330-enol				
Lemna gibba	7 d, static	metabolite	KIIA 8.6/02	E_rC_{50} 19.3 mg p.m./L[2]

[1] based on mean measured concentrations
[2] based on nominal initial concentrations
p.m. = pure metabolite

The following TER values for *Lemna gibba* exposed to spirotetramat and its metabolite BYI 08330-enol are calculated in this Tier 1 risk assessment using the maximum initial PEC_{SW} as shown in Table IIIA1 10.2.1-1.

Table IIIA1 10.8.2-2: TER for non-target aquatic plants exposed to spirotetramat and the metabolite BYI 08330-enol

Test Organism, time scale	Test substance	Ecotoxicological endpoint	PEC$_{max}$ [mg/L]	TER	Refinement required?
Lemna gibba 7 d, static-renewal	a.s.	E_rC_{50} 6.21 mg a.s./L	0.0116	535	No
Lemna gibba 7 d, static	-enol	E_rC_{50} 19.3 mg p.m./L	0.0156	1,237	No

The Tier 1 TER values for effects on non-target aquatic plants to spirotetramat and its metabolite BYI 08330-enol do not indicate an unacceptable risk according to Annex VI of the EU-directive 91/414/EEC (TER ≥ 10). Therefore it can be concluded that no adverse effects on aquatic plants are to be expected from the use of Spirotetramat OD 150 according to the proposed use pattern.

IIIA1 10.8.2.1 Aquatic plant growth – Lemna

Since Spirotetramat OD 150 acts as an insecticide, tests with the product on aquatic plants are not required. For results with the active substance spirotetramat see KIIA 8.6/01.

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IIIA1 10.8.2.2Aquatic field testing

Not required based on the findings presented and evaluated above.

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IIIA1 10.9 Effects on other non-target organisms believed to be at risk

IIIA1 10.9.1 Summary of preliminary data: biological activity & dose range finding

The data presented above allow a complete assessment of the product concerning the risk to non-target organisms, therefore no further data are considered necessary. Data on the efficacy are presented in the relevant chapter about the efficacy of the product.

IIIA1 10.9.2 Assessment of relevance to potential impact on non-target species

A risk assessment concerning the potential impact of the product on non-target species has been presented in the chapters before.

IIIA1 10.10 Other/special studies

In view of the findings above additional studies are not deemed necessary.

IIIA1 10.10.1 Other/special studies - laboratory studies

In view of the findings above additional studies are not deemed necessary.

IIIA1 10.10.2 Other/special studies - field studies

In view of the findings above additional studies are not deemed necessary.

IIIA1 10.11 Summary and evaluation of points IIIA1 9 and IIIA1 10.1 to 10.10

IIIA1 10.11.1 Predicted distribution and fate in the environment and time courses

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.

Predicted environmental concentrations in soil (PEC_{Soil}) were calculated for the 0 to 5-cm soil layer for spray application in citrus. They amounted to max. 0.115, 0.0934, 0.031 and 0.005 mg/kg soil for parent compound, BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide, respectively.
The PEC_{Soil} calculated for the 0 to 5-cm soil layer in case of spray application in lettuce were much lower, always. They amounted to max. 0.029, 0.024, 0.009 and 0.001 mg/kg soil for parent compound, BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide, respectively.

For all relevant FOCUS scenarios the leaching simulations for BYI08330 and its before mentioned metabolites resulted in predicted environmental concentrations in groundwater (PEC_{gw}) < 0.001 µg/L. Thus it can be concluded that Spirotetramat applications in citrus and lettuce in Europe are highly unlikely to cause groundwater concentrations above the limit value of 0.1 µg/L.

The maximum predicted environmental concentrations in surface water and sediment (PEC_{SW} and PEC_{Sed}) of BYI08330 calculated according FOCUS STEP 3 & 4 for use in citrus amounted to 8.43 µg/L

and 4.16 µg/kg (Thiva ditch), and 6.51 µg/L and 0.75 µg/kg (Roujan ditch), respectively. Considering a 5-m buffer zone, the respective values can be reduced to 5.86 µg/L and 2.92 µg/kg in the Thiva ditch, respectively. All the respective figures in leafy vegetables (i.e. lettuce) were much lower.
The maximum PEC_{SW} and PEC_{Sed} of the metabolites of BYI08330 were calculated according FOCUS STEP 2. Again, the use in citrus was the worst case. Maximum PEC_{SW} and PEC_{Sed} were 15.58 µg/L and 8.00 µg/kg for BYI08330-enol, and 8.59 µg/L and 5.34 µg/kg for BYI08330-ketohydroxy.
For the natural water phototransformation, products BYI08330-methoxy cylohexanone and BYI08330-methoxy cylohexylamino carboxylic acid the max. PEC_{SW} and PEC_{Sed} amounted to 1.39 µg/L and 0.04 µg/kg, and 1.21 µg/L and 0.12 µg/kg, respectively.

IIIA1 10.11.2 Non-target species at risk and extent of potential exposure

According to Council Directive 97/57/EC of 22. September 1997 establishing Annex VI to Directive 91/414/EEC, taking into account the relevant guidance documents, the following can be concluded from the available data:

Terrestrial Vertebrates
The risk assessment for terrestrial vertebrates was conducted according to the recommendations of the final version of the EU Guidance Document for Risk Assessment for Terrestrial Vertebrates (SANCO/4145/2000). The acute and short-term risk assessment indicated no unacceptable risk for birds even under the worst-case assumptions of the Tier 1 risk assessment. The trigger was not met in the conservative Tier 1 risk assessment for long-term exposure. However, considering more realistic exposure scenarios it could be shown in a refined risk assessment that no unacceptable risk for birds is given under practical field conditions.
For wild mammals, no unacceptable acute effects are expected according to the results of the conservative Tier 1 risk assessment.

Aquatic Organisms
The risk assessment for terrestrial vertebrates was conducted according to the recommendations of the final version of the EU Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev.4). The TER values for acute and chronic exposure of aquatic organisms were met in nearly all cases in the conservative Tier 1 risk assessment on the basis of worst-case laboratory studies. Only for the acute exposure of *Chironomus riparius* to the product Spirotetramat OD 150 applied in citrus the trigger was not met. However, it was shown that a buffer zone of 5 m will be sufficient to reach the trigger value defined in Annex VI of Directive 91/414 EEC. Thus, no unacceptable risks for aquatic organisms are to be expected from the use of Spirotetramat OD 150 under practical field conditions.

Honey bees
The Q_{HO} and the Q_{HC} values are substantially below 50, indicating, that at the maximum recommended field rate an unacceptable risk to honeybees is not expected. In a brood feeding test effects were detected after providing a sugar solution containing 0.0144% of the test item to bee colonies. However, under more realistic exposure conditions, in a semi-field brood test no treatment-related effects to bee brood and colony development were found. This was confirmed by an additional field test where no adverse effects of the compound could be seen neither in brood development or colony condition, nor on any other parameter assessed, such as mortality or foraging activity. Therefore, applications of Spirotetramat according to the submitted GAP can be considered safe to foraging bees as well as to bee brood.

Terrestrial Non-Target Arthropods
The tier I risk assessment indicated a potential risk for in-field and off-field non-target arthropods. Based on the results of the laboratory and extended laboratory studies, *Typhlodromus pyri* has been identified as the most sensitive indicator species. However, the results of an aged residue study and a field trial showed that under realistic field conditions non-target arthropods will not be significantly harmed at all

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by an application according to the use patterns and the potential for recovery within one season was shown.
Thus it can be concluded that the use of Spirotetramat OD 150 will not pose unacceptable risk to non-target arthropod populations under field conditions.

Soil Macroinvertebrates
Spirotetramat and its metabolites BYI 08330-enol and BYI 08330-cis-ketohydroxy have no negative influence on earthworms as shown by the acute Tier 1 risk assessment. Thus, no unacceptable risk to soil non-target macro-organisms is to be expected from the use of Spirotetramat OD 150.

Soil Microorganisms
Spirotetramat has no negative influence on the turnover of organic carbon and nitrogen in soil. Thus, no unacceptable risk to soil non-target micro-organisms is to be expected from the use of Spirotetramat OD 150 under practical field conditions.

Non-Target Terrestrial Plants
In Tier 1 seedling emergence and growth studies with Spirotetramat OD 150, no pronounced phytotoxic effects were observed at application rates up to 288 g a.s./ha. On the other hand, in vegetative vigour tests effects were evident and the lowest ER_{50} determined was 134 g a.s./ha. Based on this value, it was shown that by using 50% drift reducing equipment or a 5 m in-crop buffer a TER of >5 can be achieved for citrus. However, using the ER_{50} of 149.2 g a.s./ha from a higher tier semi-field study a refined TER of 3.5 for the most sensitive species was calculated. Since this value exceeds the refined TER trigger of 2, no mitigation beyond the standard 3m buffer is considered necessary for the use of the product in citrus. For the use in lettuce the TER is well in excess of 5 at the standard 1 m buffer.

IIIA1 10.11.3 Short and long term risks for non-target organisms

The available toxicity data and the relevant exposure data as combined in the respective risk assessments for terrestrial vertebrates, aquatic organisms, honeybees, non-target arthropods, earthworms & soil macro-organisms, soil micro-organisms and non-target terrestrial plants indicate that no adverse short-term or long-term effects on these species are to be expected from the use of Spirotetramat OD 150 according to the proposed use pattern.

IIIA1 10.11.4 Risk of fish kills and fatalities in large vertebrates

For fish it can be concluded, that the application of the product Spirotetramat OD 150 according to the proposed use pattern and use conditions will not result in unacceptable adverse effects.

IIIA1 10.11.5 Precautions necessary to avoid or minimize contamination

No unacceptable risk to non-target organisms is to be expected from the application of Spirotetramat OD 150 according to the intended use patterns when appropriate risk mitigation measures as described above are applied.

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