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Final Report

Laboratory Testing for Toxicity (Acute Oral LD_{50}) of WAK 3839 on Honey Bees (*Apis mellifera* L.) (Hymenoptera, Apidae) -Limit Test-

(GLP compliant study based on EPPO 170 (1992))

Author: Dipl. Biol. [redacted]

Study Completion Date: December 20, 1999

Sponsor

Bayer AG
Crop Protection Division
Institute for Environmental Biology
Alfred-Nobel-Str. 50
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Germany

Test Facility

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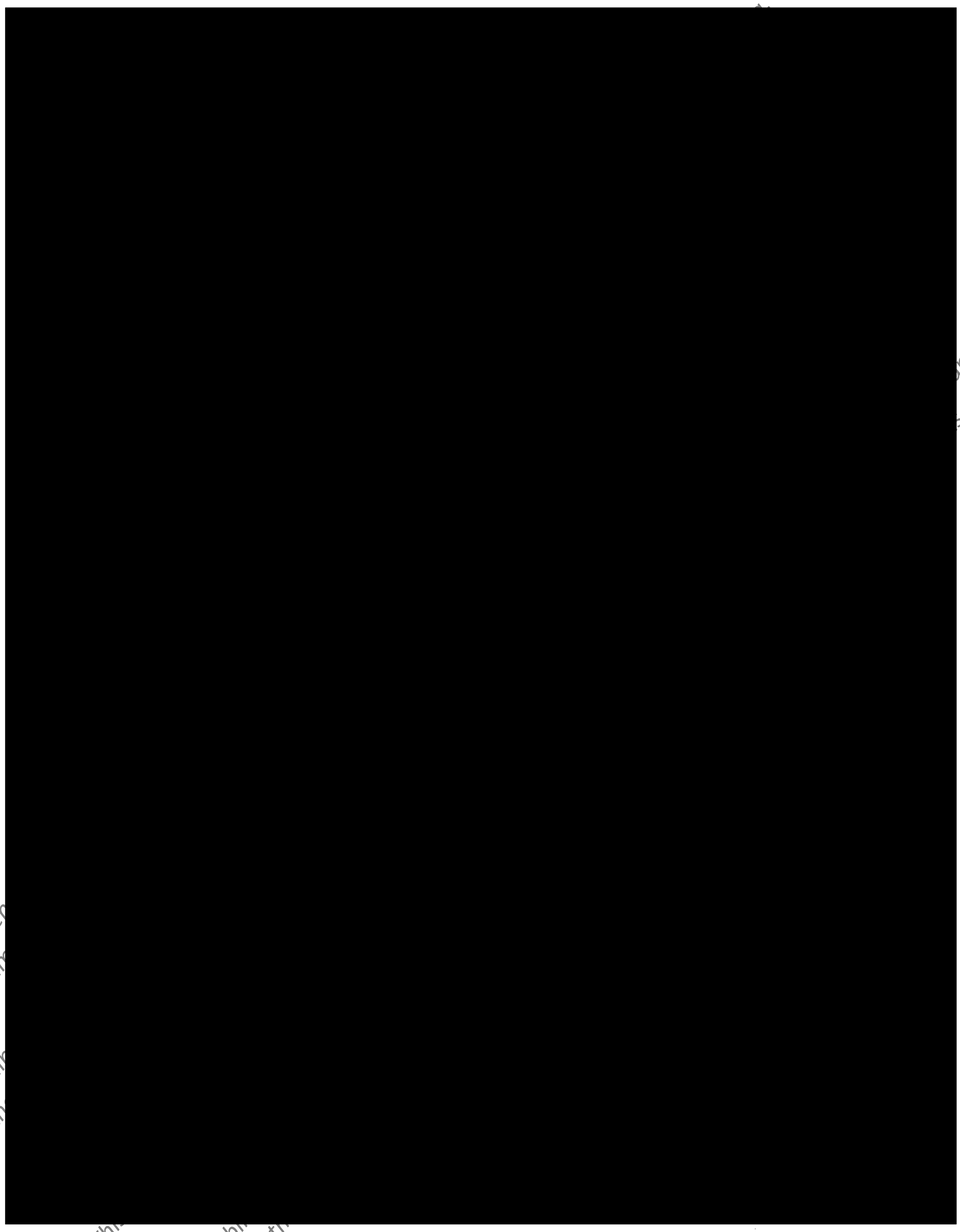
Project 6390036

6390036 / MO-00-000582

Copy of GLP Certificate



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1. Summary

Report: [redacted] (1999): Laboratory Testing for Toxicity (Acute Oral LD_{50}) of WAK 3839 on Honey Bees (*Apis mellifera* L.) (Hymenoptera, Apidae) -Limit Test-
Source: IBACON, unpublished report No.: 6390036, December 20, 1999.

Guidelines: EPPO No. 170
Deviations: temperature: 28 - 29 °C; relative humidity: 52 - 86 % instead of 25 ° C ± 2 ° C and relative humidity of 60 -70 % as indicated in the guideline

GLP: yes (certified laboratory)

Material and methods: test substance: WAK 3839, purity: 99 %, batch number: 950411ELB02, (test substance was obtained in a 0.1 % ethanol solution; WAK 3839 was extracted by evaporating of the ethanol solution); under laboratory conditions, starved honey bees (*Apis mellifera*, 3 groups of 10 bees per dose) received a single oral dose of either 21.8, 0.3 or 0.08 µg per bee in 4-19 mg sugar solution. Subsequently, honey bees were observed over a period of 96 hrs for behavioural impairments and survival rate. The test was prolonged up to 96 hours because of increasing mortality between 24 and 48 hours at 0.08 µg/bee. The reference treatment (0.2 µg dimethoate per bee) caused a 100 % mortality (the facility-specific LD_{50} dose for dimethoate is typically between 0.10 and 0.14 µg/bee).

Findings: Toxicity to Honey Bees, Laboratory Tests

Test substance	WAK 3839
Test object	*Apis mellifera*
Application rates µg product/bee	21.8*, 0.3* and 0.08*
Exposure	oral (sugar solution)
LD_{50} µg/bee (48 and 96h)	ca. 0.08

* values based on actual intake of the test substance

Observations: the observation period was extended for 48 hours because of delayed mortality in the lowest dose groups. Although bees were previously starved for 60 minutes the bees ingested only 22, 30 and 80 % of the provided sugar solution in dosing group of 100, 1 and 0.1 µg/bee, respectively. This food rejection indicates a strong antifeedant effect of the test substance. In the highest dosage groups, immediately after uptake, the bees were strongly affected (apathy, discoordinated movements). Therefore, the bees were unable to take in the whole amount of offered contaminated food. Oral doses of 21.8 µg/bee and 0.3 led to 100 % mortality during the first 4 and 24 hours, respectively. After application of an oral dose with 0.08 µg WAK 3839 per bee, 46.7 % of the bees died during 48 hours after the application. No further mortality occurred during 72 and 96 hours. Behavioural impairments like discoordinated movement and apathy in this dose group were observed for the first 24 hours. No more behavioural abnormalities occurred until the end of the experiment.
No bee died in neither the acetone, nor in the pure syrup control. All bees died after treatment with Dimethoate.

2. Survey of the Study

2.1 General Information

Title:	Laboratory Testing for Toxicity (Acute Oral LD_{50}) of WAK 3839 on Honey Bees (*Apis mellifera* L.) (Hymenoptera, Apidae) - Limit Test -
Sponsor:	Bayer AG Crop Protection Division Institute for Environmental Biology Alfred-Nobel-Str. 50 40789 Monheim Germany
Monitoring:	[redacted]
Test Substance:	WAK 3839
Test Facility:	Institut für Biologische Analytik und Consulting IBACON GmbH Arheilger Weg 17 64380 Rossdorf Germany
IBACON Project:	6390036

Project Staff:

Test Facility Management:	[redacted]
Study Director:	Dipl. Biol. [redacted]
Technical Coordination:	[redacted]
Head of Quality Assurance Unit (QAU):	Dipl. Biol. [redacted]
Quality Assurance Unit Manager:	Dipl. Biol. [redacted]

Schedule:

Study Initiation Date:	September 13, 1999
Experimental Starting Date:	September 16, 1999
Experimental Completion Date:	September 20, 1999
Draft Report Date:	December 3, 1999
Study Completion Date:	December 20, 1999

2.2 Good Laboratory Practice

This study was performed in compliance with:

- The OECD Principles of Good Laboratory Practice (as revised in 1997) and the
- Chemikaliengesetz ('*Chemicals Act*') der Bundesrepublik Deutschland (ChemG), Anhang 1 ('*Annex 1*'), 1994/97.

This study was assessed in compliance with the study protocol and the IBACON Standard Operating Procedures. This study and/or test facility were periodically inspected by the Quality Assurance Unit (QAU) and the dates and the phases of the inspections are included in this final report. The data contained within this final report were audited in comparison to the raw data.

A quality assurance statement, signed by the Quality Assurance Unit, is included in this final report.

2.3 Archiving

The following data / sample(s) will be archived

for 15 years:
- all raw data
- the study protocol
- one certified copy of the final report

for at least 2 years:
- one sample of the test substance and of the toxic standard

following the date on which the final report is audited by the Quality Assurance Unit at:

Institut für Biologische Analytik
und Consulting IBACON GmbH
Arheilger Weg 17
64380 Rossdorf
Germany

No raw data or material relating to the study will be discarded without the sponsor's prior consent.

2.4 Signatures

Study Director: Dipl. Biol. [redacted]

date: December 20, 1999

Test Facility Management: [redacted]

date: December 20, 1999

3. Quality Assurance Unit Statement

[redacted]

4. Statement of Compliance

[redacted]

5. Objectives of the Study

5.1 Title

Laboratory Testing for Toxicity (Acute Oral LD_{50}) of WAK 3839 on Honey Bees (*Apis mellifera* L.) (Hymenoptera, Apidae) - Limit Test -

5.2 Purpose

If honey bees can be exposed to residues of WAK 3839, this study is useful for the registration of the pesticides in question. This study provides:

- the acute toxicity levels of the test substance to honey bees;
- toxicity information comparable to expected residues from standard rates, for assessment of the potential hazard to honey bees;
- informational support for precautionary label statements.

5.3 Guidelines/Recommendations

This study was designed to comply with the following method:

- EPPO 1992: Guideline on test methods for evaluating the side-effects of plant protection products on honey bees, Bulletin OEPP/EPPO Bulletin 22, 203-215 1992, No. 170

5.4 Justification for the Selection of the Test System

This test system is required by the Registration Directive 91/414/EEC and/or by the 'SETAC - Guidance document on regulatory testing procedures for pesticides with non-target arthropods' (Barrett et al. 1994) for the hazard assessment of pesticides.

6. Materials and Methods

6.1 Test Substance, Control and Toxic Standard

Test Substance

The test substance and the information concerning the test substance were provided by the sponsor:

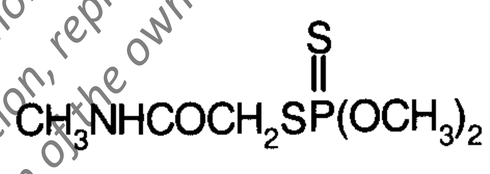
Name:	WAK 3839
Batch No.: (Lot No.:)	950411ELB02
Active Ingredient(s)/Purity:	99 % according to the sponsor and the Certificate of Analysis
Certificate of Analysis/ dated and signed:	May 25, 1999, by [redacted]
Indication:	insecticide
Aggregate State at Room Temperature:	solid
Colour:	yellowish
Solubility:	in acetone: not indicated
Stability:	pure: see expiry date in acetone: not indicated
Expiry Date:	5/2004
Storage:	in original container, cold, in the dark

Controls

Controls:	1) pure syrup 2) acetone + syrup

Toxic Standard

The information concerning the toxic standard according to the substance container label:

Name:	Perfekthion EC
Batch No.:	98-1
Active Ingredient/Purity:	Dimethoate: 396 g/L
Chemical Structure of a.i.:	
Manufacturer:	BASF AG, Unternehmensbereich Pflanzenschutz, D-67056 Ludwigshafen
Expiry Date:	October/2000
Storage:	at room temperature, in the dark, in original container
Amount Applied in this Study:	0.2 µg active ingredient per bee

6.2 Test System

Taxonomic Group:	worker honey bees (Insecta, Hymenoptera)
Species:	adult *Apis mellifera carnica* L.
Age and Sex:	4 - 6 week old female bees
Origin:	honey bee colonies, disease-free and queen-right, bred by IBACON
Collection:	with glass tubes, from the flight board without anaesthetics
Bee Maintenance:	conformed to proper cultural practices

6.3 Test Units

Type:	stainless steel chambers
Size:	10 cm x 8.5 cm x 5.5 cm (length x width x height)
Front Side:	removable glass sheet
Bottom:	perforated with 98 ventilation holes; ø 1 mm
Inner Walls:	lined with filter paper (Co. Macherey & Nagel, D-52355 Düren, Art. No. 68)
No. Of Individuals:	10 per test unit
Replicates:	3 per dosage/control
Identification:	test units were uniquely identified with study number, application date, treatment, concentration and replicate number
Bee Assignment:	assigned to test levels impartially

6.4 Test Conditions

Surrounding Type:	incubators
Temperature:	28-29 °C[1]
Relative Humidity:	52 - 86 %[1]
Light:	darkness (except during observation)
Ventilation:	ventilation to avoid possible accumulation of pesticide vapour
Recording:	test conditions were recorded with suitable instruments and documented in the raw data

6.5 Food

Food:	commercial ready-to-use syrup (Apiinvert; 30 % Saccharose, 31 % Glucose, 39 % Fructose) *ad libitum*

[1] in deviation from the guidelines which recommend a temperature of 25 ° C ± 2 ° C and a relative humidity of 60 - 70 %. Experience of IBACON has shown that this deviation to the guideline will have no adverse effect on the study.

6.6 Application of the Test Substance, the Control and the Toxic Standard

The dosage applied was not adjusted to reflect the percentage a.i.

The procedure of preparation of the test substance solution is described in detail in the Attachment, page 22.

Application in the Oral Test:	4 - 19 mg of WAK 3839 contaminated food (for the foreseen 100 µg/bee nominal dose the test substance was distributed directly in the food, for the 1 and 0.1 µg/bee nominal dosages 1 part solvent (=acetone) including the test substance + 19 parts syrup were used) offered in syringes which were weighed before and after introduction into the cages (duration of uptake did not exceed 3 hours)
Measured Dosages of the Test Substance in the Oral Test:	21.8, 0.3 and 0.08 µg/bee (values based on actual intake of the test substance)
Dosage of the Toxic Standard:	0.2 µg a.i. Dimethoate per bee
Controls:	1) WAK 3839 free sugar solution (used as the control for the 21.8µg/bee dosage group) 2) WAK 3839 free sugar solution with acetone as solvent (used as the control for the 0.3µg and 0.08µg/bee dosage group)

6.7 Course of the Test

Treatment Groups:	controls, 3 dosages of test substance, toxic standard
Replicates:	3 per treatment group
Individuals:	10 per unit, 30 individuals per treatment group
Starvation Time:	60 minutes
Exposure Time:	96 hours (experiment was prolonged for further 48 hours, because of increasing mortality in the 0.08 µg/bee dosing group between 24 and 48 hours)

6.8 Test Parameters

Mortality:	number of dead bees after 60 minutes; 2, 4 hours (first day); 24, 48, 72 and 96 hours
Behavioural Abnormalities:	behavioural abnormalities (vomiting, apathy, intensive cleaning, discoordinated movements) after 60 minutes; 2, 4 hours (first day); 24, 48, 72 and 96 hours

6.9 Result Evaluation

Mortality:	results obtained from the bees treated with test substance are compared to those obtained from the toxic standard and the controls. Due to the results it was not necessary to conduct statistical analysis.

6.10 Validity Criteria of the Study

Control Mortality:	no control mortality at experimental end (96 hours)
Toxic Standard Mortality:	resulted in 100 % mortality

6.11 Deviations to the Study Protocol

Concerning:	dosages of the test substance
According to the Study Protocol:	two dosages should be tested (100 and 1 µg/bee)
Deviation to the Study Protocol:	three dosages were tested
Reason for the Deviation:	because, in the two highest nominal dosage groups all bees were strongly affected during the first hour of the experiment, one additional dosage group was prepared in order to enable a broader mortality data base
Presumed Effect on the Study:	an additional dosage group improved the study design; the scientific base for the test substance assessment is more quantifiable
Concerning:	Test Conditions
According to the Study Protocol:	40 - 70 %
Deviation to the Study Protocol:	the relative humidity remained in the range of 52 - 86 % instead of 40 - 70% as indicated in the study protocol
Reason for the Deviation:	because of technical reasons (climatic) the relative humidity had a broader range than expected
Presumed Effect on the Study:	none, the broader humidity range reflects natural conditions and has no adverse effect on the outcome of the study

7. Results and Discussion

7.1 Oral Toxicity Test

After a starving period of 60 minutes, all bees died after 4 hours following the ingestion of 21.8 µg WAK 3839 per bee. Oral uptake of 0.3 µg WAK 3839 led to 100 % mortality after 24 hours. Behavioural abnormalities like apathy and discoordinated movements of the surviving bees occurred in both dosing groups.

14 of the 30 bees (46.7 %) died after uptake of 0.08 µg WAK 3839 until the end of the 96 hours lasting experimental time. The mortality level of 46.7 % was reached 48 hours after application of the test substance. No more bee died between 48 and 96 hours after uptake of WAK 3839 (Table 1, Appendix Table 2 - 4).

Behavioural impairments like apathy or discoordinated movements were observed for the first 24 hours.

The LD_{50} of WAK 3839 must be estimated as 0.08 µg/bee.

Two different controls were used for this test:

The pure syrup control was used for the 21.8 µg/bee group, because the test substance was mixed directly in the syrup. In the 0.3 and 0.08 µg/bee dosage groups, acetone was used as solvent, respectively.

No mortality occurred in the control groups within the whole experiment.

100 % mortality occurred after ingestion of 0.2 µg Dimethoate per bee in the toxic standard group.

Table 1. Mortality[a] and behavioural abnormalities[a] of the bees in the oral toxicity test [b]

	after 1 hour		after 4 hours		after 24 hours		after 48 hours		after 72 hours		after 96 hours	
uptaken test substanc µg/bee	mortality mean %	behav. abnorm. mean %	mortality mean %	behav. abnorm. mean %	mortality mean %	behav. abnorm. mean %	mortality mean %	behav. abnorm. mean %	mortality mean %	behav. abnorm. mean %	mortality mean %	behav. abnorm. mean %
21.8	93.3	6.7	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
0.3	53.3	46.7	83.3	16.7	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
0.08	0.0	96.7	13.3	86.7	36.7	10.0	46.7	0.0	46.7	0.0	46.7	0.0
syrup	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
solvent	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
toxic st.	0.0	0.0	76.7	23.3	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0

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

[b] see Appendix for details

behav. abnorm. = behavioural abnormalities; syrup = pure syrup control; solvent = acetone control; toxic st. = toxic standard

7.2 Conclusions

Mortality:	Oral $LD_{50\ (48\ and\ 96h)}$ of WAK 3839: ca. 0.08 µg/bee
Behavioural Abnormalities:	Behavioural abnormalities occurred in all dosage groups (apathy, discoordinated movements).

8. References

Barrett K.L., Grandy N., Harrison E.G., Hassan S.A. & Oomen P. 1994: SETAC - Guidance document on regulatory testing procedures for pesticides with non-target arthropods. 28-30 March 1994, IAC Wageningen, The Netherlands

Chemikaliengesetz der Bundesrepublik Deutschland (ChemG), Anhang 1, in der Fassung der Bekanntmachung vom 25. Juli 1994 (BGBl. I S. 1703) mit Änderungen vom 27. September 1994 (BGBl. I S. 2705) und 14. Mai 1997 (BGBl I S. 1060)

EC Agrochemical Registration Directive (DS65) (Directive 91/414/EEC). ANNEX III. Requirements for the dossier to be submitted for the authorization of a plant production product

EPPO 1992: Guideline on test methods for evaluating the side-effects of plant protection products on honey bees, Bulletin OEPP/EPPO Bulletin 22, 203-215 1992, No. 170

OECD Principles of Good Laboratory Practice, adopted by Council on 26th November 1997 [C(97)186/Final], Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998

9. Distribution of the Final Report

Sponsor:	1x (the original final report)
IBACON:	1x (one certified copy of the original final report)

Appendix

Table 2. (Exact Data). Definitive oral toxicity test; mortality and behavioural abnormalities of the bees

	test substance	after 1 hour			after 2 hours			after 4 hours			after 24 hours			after 48 hours		
unit #	dosage µg/bee	dead #	beh. #	abnor. symp.	dead #	beh. #	abnor. symp.	dead #	beh. #	abnor. symp.	dead #	beh. #	abnor. symp.	dead #	beh. #	abnor. symp.
1	19.0	10	0		10	0		10	0		10	0		10	0	
2	18.5	8	2	c,b	8	2	c,b	10	0		10	0		10	0	
3	28.0	10	0		10	0		10	0		10	0		10	0	
1	0.3	6	4	c	9	1	c	9	1	c	10	0		10	0	
2	0.3	5	5	c	8	2	c	8	2	c	10	0		10	0	
3	0.3	5	5	c	7	3	c	8	2	c	10	0		10	0	
1	0.09	0	10	c	0	9	b,c	2	8	b,c	4	1	b	5	0	
2	0.07	0	10	c	0	10	b,c	0	10	b,c	4	1	b	5	0	
3	0.08	0	9	c	0	10	b,c	2	8	b,c	3	1	b	4	0	
	syrup															
1	control	0	0		0	0		0	0		0	0		0	0	
2	control	0	0		0	0		0	0		0	0		0	0	
3	control	0	0		0	0		0	0		0	0		0	0	
	solvent															
1	control	0	0		0	0		0	0		0	0		0	0	
2	control	0	0		0	0		0	0		0	0		0	0	
3	control	0	0		0	0		0	0		0	0		0	0	
	toxic															
1	standard	0	0		0	4	b,e	7	3	b,c	10	0		10	0	
2	standard	0	0		4	6	b,e	7	3	c	10	0		10	0	
3	standard	0	0		1	6	b,e	9	1	e	10	0		10	0	

# = number of individuals; beh. abnor. = behavioural abnormalities
symp. = observed symptoms according to the following key: a= food refusal/vomiting; b = moving coordination problems;
c = apathy; d= intensive cleaning; e=nervous; f = sitting in one corner of the chamber
syrup control = pure syrup control; solvent control = acetone control

Table 2. (Exact Data, continued). Prolongation of the oral test up to 96 hours; mortality and behavioural abnormalities of the bees

unit	test substance dosage[a] µg/bee	72 hours dead #	72 hours beh. abnor. #	72 hours beh. abnor. symp.	96 hours dead #	96 hours beh. abnor. #	96 hours beh. abnor. symp.
1	19.0	10	0		10	0	
2	18.5	10	0		10	0	
3	28.0	10	0		10	0	
1	0.3	10	0		10	0	
2	0.3	10	0		10	0	
3	0.3	10	0		10	0	
1	0.09	5	0		5	0	
2	0.07	5	0		5	0	
3	0.08	4	0		4	0	
	syrup						
1	control	0	0		0	0	
2	control	0	0		0	0	
3	control	0	0		0	0	
	solvent						
1	control	0	0		0	0	
2	control	0	0		0	0	
3	control	0	0		0	0	
	toxic standard						
1	standard	10	0		10	0	
2	standard	10	0		10	0	
3	standard	10	0		10	0	

= number of individuals; beh. abnor. = behavioural abnormalities
[a] dosages calculated after reweighing the syringes
symp. = observed symptoms according the following key: a=vomiting
b = moving coordination problems; c = apathy; d= intensive cleaning
syrup = pure syrup control; solvent = acetone control

Table 3. (Relative Data). Definitive oral toxicity test; mortality and behavioural abnormalities of the bees

test substance dosage	after 1 hour				after 4 hours				after 24 hours				after 48 hours			
	mortality		beh.abnor.[a]		mortality		beh.abnor.[a]		mortality		beh.abnor.[a]		mortality		beh.abnor.[a]	
µg/bee	mean %	±SD %	mean %	±SD %	mean %	±SD %	mean %	±SD %	mean %	±SD %	mean %	±SD %	mean %	±SD %	mean %	±SD %
21.8	93.3	11.5	6.7	11.5	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
0.3	53.3	5.8	46.7	5.8	83.3	5.8	16.7	5.8	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
0.08	0.0	0.0	96.7	5.8	13.3	11.5	86.7	11.5	36.7	5.8	10.0	0.0	46.7	5.8	0.0	0.0
syrup	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
solv.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
toxic st.	0.0	0.0	0.0	0.0	76.7	11.5	23.3	11.5	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0

[a]beh. abnor. = behavioural abnormalities; mean = mean of three replicates; ±SD = standard deviation from three replications
syrup = pure syrup treated control; solv. = acetone control; toxic st. = toxic standard

Table 3. (Relative Data, continued). Prolongation up to 96 hours Definitive oral toxicity test

test substance dosage	72 hours				96 hours			
	mortality		beh.abnor.[a]		mortality		beh.abnor.[a]	
µg/bee	mean %	±SD %	mean %	±SD %	mean %	±SD %	mean %	±SD %
21.8	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
0.3	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
0.08	46.7	5.8	0.0	0.0	46.7	5.8	0.0	0.0
syrup	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
solvent	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
toxic st.	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0

[a] beh. abnor. = behavioural abnormalities; mean = mean of three replicates;
syrup = pure syrup control; solv. = acetone control; toxic st. = toxic standard
±SD = standard deviation from three replications

Table 4. Definitive oral intake

substance dosage nominal	substance concentrations in food µg / mg	weight of syringes start[a] mg	end[b] mg	difference mg	uptaken solution mg / bee	uptaken substance µg / bee[d]	ø[c] µg / bee
100 µg/bee							
1	5	11187	11149	38	4	19.0	
2	5	11130	11093	37	4	18.5	21.8
3	5	11275	11219	56	6	28.0	
1 µg/bee							
1	0.05	11201	11138	63	6	0.3	
2	0.05	11202	11149	53	5	0.3	0.3
3	0.05	11236	11167	69	7	0.3	
0.1 µg / bee							
1	0.005	11207	11018	189	19	0.09	
2	0.005	11204	11070	134	13	0.07	0.08
3	0.005	11075	10925	150	15	0.08	
syrup control							
1	0	11208	10952	256	26	0.0	
2	0	11215	10957	258	26	0.0	0.0
3	0	11317	11069	248	25	0.0	
acetone control							
1	0	11242	11001	241	24	0.0	
2	0	11201	10957	244	24	0.0	0.0
3	0	11258	11026	232	23	0.0	
toxic standard							
Dimethoate							
0.2 µg/bee							
1	0.01	11227	10978	249	25	0.2	
2	0.01	11260	11027	233	23	0.2	0.2
3	0.01	11154	10905	249	25	0.2	

[a] weight of syringes at the start of the experiment, [b] after removing from the test cages;
[c] ingested solution as calculated average, [d] results are rounded results, calculated from the exact data

Attachment: Preparation of the Test Substance S itions

Preparation of the test substance:

For the experiment the test substance was delivered by the sponsor as a dilu on of 0.1 % WAK 3839 in pure analytical ethanol. The ethanol was completely evaporated in order to ceive the pure test substance from the dilution. The evaporation was done in a porcelain dish ler room temperature until the solvent was completely disappeared. After complete evaporation yellow h-violet crystals were obtained.

Oral Test

100 µg/bee dosage (nominal):

20 mg of the test substance was mixed with 4 g syrup and overnight stirred RT until the application.

Control: pure syrup.

1 µg/bee dosage (nominal):

50 mg of the test substance was dissolved ad 50 g acetone (stock solution) eated with ultra sonic for 20 minutes and overnight stored in a refrigerator until the application. ortly after stirring the test substance started to sedimentize. Therefore, the stock solution was taken o under permanent stirring.

500 mg of the stock solution was added to 9.5 g syrup (1:20).

Control: 0.5 g acetone + 9.5 g syrup (1:20)

0.1 µg/bee dosage (nominal):

the 0.1 µg / bee concentration was obtained by diluting the 1 µg/bee dilutic 1 : 10.

500 mg of the 1:10 dilution was added to 9.5 g syrup (1:20) for prepar on of the 0.1 µg/bee dosage group.

Control: 0.5 g acetone + 9.5 g syrup (1:20)

100, 1 and 0.1 µg/bee should be obtained when 20 mg of the contaminat sugar solution per bee were administered.

21.8, 0.3 and 0.08 µg/bee were obtained, because the bees ingested betw 1 4 and 19 mg contaminated food per bee.