Institut für
Biologische Analytik
und Consulting GmbH

Study Report

Laboratory testing for toxicity (acute contact and oral LD_{50}) of Confidor WG 70 to honey bees (*Apis mellifera L.*) (Hymenoptera, Apidae)

(GLP compliant study according to EPPO 170 (1992))

Author: Dipl. Biol. [redacted]

Study report date: October 4, 1995

Study performed for Sponsor:

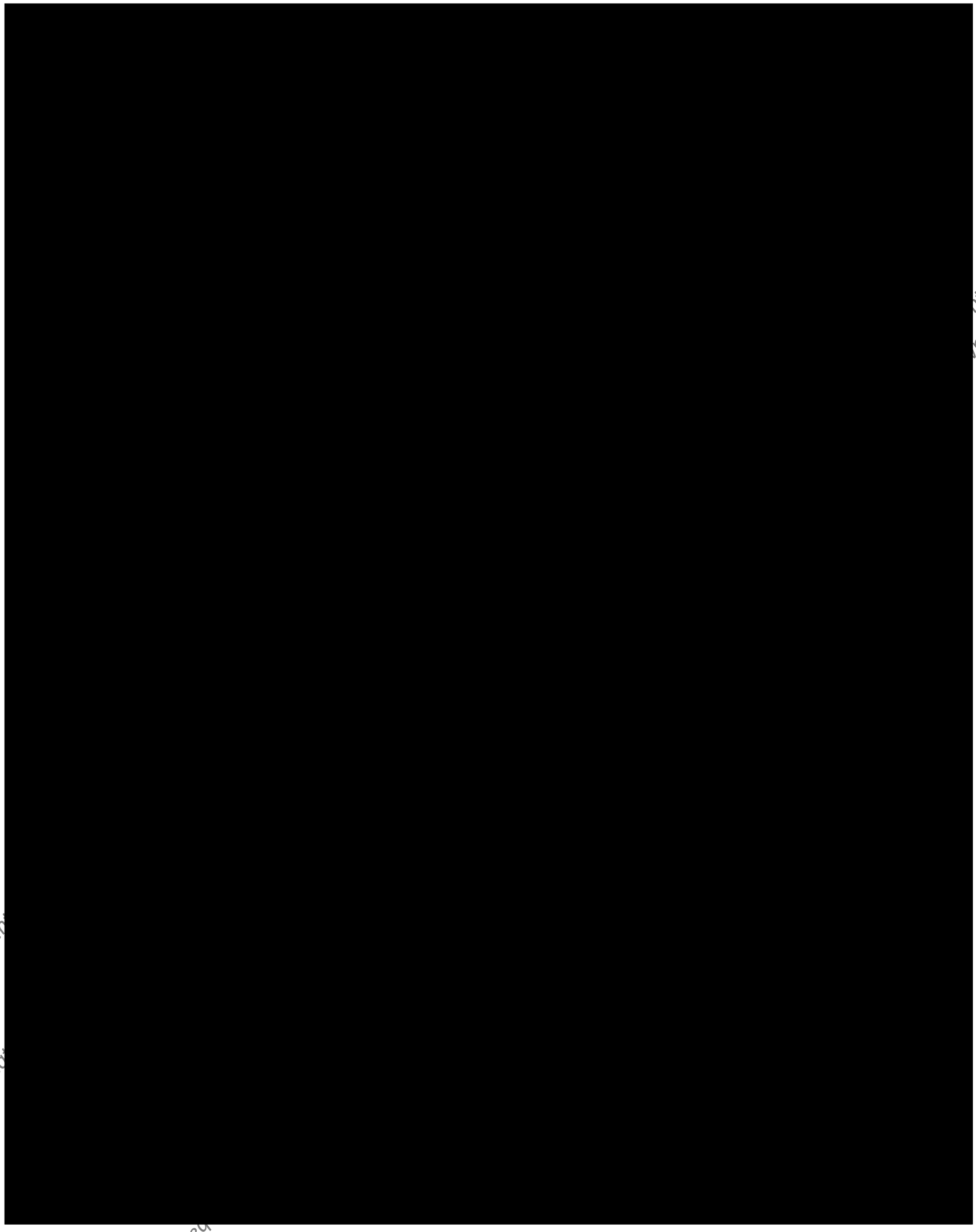
Bayer AG
Pflanzenschutz
Ökobiologie
D-51368 Leverkusen

Testing facility

Institut für
Biologische Analytik
und Consulting IBACON GmbH
D - 64380 Rossdorf

Project 780036

Copy of GLP Certificate



Contents

List of tables

List of figures

1. Summary

The contact and oral LD_{50} (24 h and 48 h) of Confidor WG 70 to honey bees were tested according to EPPO 170 (1992) and GLP regulations. Confidor WG 70 was applied in five dosages (contact and oral toxicity test), one solvent control, one untreated negative control (contact test) and one positive control with toxic standard (Dimethoate 0.2 µg a.i./bee). The following dosages of the test substance were tested in three replicates of ten bees each:

Contact toxicity test			Oral toxicity test		
Test Substance Dosage (nominal) µg / bee	Mortality 24 h (%)	Mortality 48 h (%)	Test Substance Dosage µg / bee	Mortality 24 h (%)	Mortality 48 h (%)
1.0	30.0	76.7	0.085	36.7	76.7
0.5	13.3	60.0	0.017	53.3	56.7
0.1	20.0	23.3	0.0088	40.0	40.0
0.05	3.3	3.3	0.0017	0.0	6.7
0.01	0.0	0.0	0.0009	0.0	0.0
calculated LD_{50} µg/bee (95 % cofidence limits)	> 1	0.35 (0.25 to 0.51)	calculated LD_{50} µg/bee (95 % cofidence limits)	≥ 0.085	0.0167 (0.0105 to 0.0264)
Controls	Mortality 24 h (%)	Mortality 48 h (%)	Controls	Mortality 24 h (%)	Mortality 48 h (%)
untreated Control	0.0	6.7	-	-	-
solvent control	0.0	3.3	negative control	0.0	3.3
positive control	96.7	96.7	positive control	96.7	100.0

The results of this study show toxic effects of Confidor WG 70 to honey bees in the contact and oral toxicity test. The acute contact LD_{50} (48h) was calculated to be 0.35 µg/bee and the acute oral LD_{50} (48h) was calculated to be 0.0167 µg/bee.

2. Survey of the study

2.1 General

Title

Laboratory testing for toxicity (acute contact and oral LD_{50}) of

Confidor WG 70 to honey bees (*Apis mellifera* L.)

(Hymenoptera, Apidae)

Sponsor:

Bayer AG

Pflanzenschutz

Ökobiologie

D-51368 Leverkusen

Monitoring: [redacted]

Test substance:

Confidor WG 70

Testing facility:

Institut für Biologische Analytik

und Consulting IBACON GmbH

Industriestrasse 1

D-64380 Rossdorf

IBACON - Projectnumber: 780036

Project staff

Management: [redacted]

Study director: Dipl. Biol. [redacted]

Quality Assurance Unit: [redacted]

Schedule

Date of protocol and German translation:	May 11, 1995
Date of 1st amendment to protocol:	June 29, 1995
Experimental start date:	July 24, 1995
Experimental termination date:	August 18, 1995
Date of draft report:	September 12, 1995
Date of report:	October 4, 1995

2.2 Quality assurance

This study was performed in compliance with:

-'The OECD principles of Good Laboratory Practice' (Paris, France 1992)

-Chemikaliengesetz (Chemicals Act) of the Federal Republic of Germany, Anlage 1 (Annex 1), dated July 25, 1994 (BGBL I 1994, p. 1703).

This study was assessed to assure compliance with the protocol and the IBACON Standard Operating Procedures. The study and/or testing facility were periodically inspected by the Quality Assurance Unit (QAU) and the dates and phases of the inspections are included with the study report. The data contained with the report were audited. A quality assurance statement, signed by the QAU-Manager, is included in the report.

2.3 Archiving

IBACON, Institut für Biologische Analytik und Consulting GmbH, Industriestrasse 1, D - 64380 Rossdorf, will archive the following data for 30 years: - all raw data, the protocol and a certified copy of the final report.

The following sample will be archived for at least 2 years following the date on which the report is audited by the Quality Assurance Unit: - sample of the test article.

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

2.4 Signatures of project staff

Study Director: Dipl. Biol. [redacted]

[redacted]

date: October 4, 1995

Management: for Dr. [redacted]

[redacted]

date: October 04, 1995

3. Quality Assurance Unit

[redacted]

4. Statement of compliance

[redacted]

5. Objectives of study

5.1 Introduction

Honey bees (*A. mellifera*) can be impacted by plant protection products. If the proposed use pattern of Confidor WG 70 indicates a possible exposure of honey bees, this study shall be useful for the registration of the plant protection products in question.

6. Guidelines

This study was based on the procedures indicated by the following internationally accepted guideline:

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

7. Material and methods

7.1 Test substance and positive control substance

Name:	Confidor WG 70
Batch - No.:	233413210
Tox-No.:	3825 - 00
Product-No.:	00 462947
Active ingredients/Purity:	69.30 % Imidacloprid
Certificate of Analysis date:	April 12, 1995
Use:	Insecticide
Expiration date:	March 23, 1996
Aggregate State at RT:	solid (granules)
Colour:	brown
Solubility in:	water: suspensible (dispersible)
Stability:	
Hydrolysis of the active ingredient:	DT_{50} of a.i. > 1 year (pH 7) DT_{50} of a.i. ca. 1 year (pH 9)
Sample received:	May 5, 1995; 12.00 a.m.
Storage:	at room temperature

Positive control substance

Name:	Perfekthion
Active Ingredient:	400 g/l (37 %) Dimethoate
Manufacturer:	BASF AG, D-67056 Ludwigshafen
Batch - No.:	98 0878
Applied Amount in this Study:	0.5 µl of formulation (corresponding to 0.2 µg active ingredient) per bee
Expiration Date:	11/1995
Storage:	in original container, protected from light at aprox. 20°C.
Safety Precautions:	routine hygienic procedures will be sufficient to assure personnel health and safety.

7.2 Test system

The study was performed with worker honey bees (*Apis mellifera* L.), about four to six weeks old, bred by IBACON in normal beekeeper's manner (responsible beekeepers: [redacted], Dipl. Biol. [redacted]). For the tests, the bees were caught randomly from the entrance hole and from the edge positioned honeycombs of the hives in groups of ten with glass capture tubes. The capture took place without anaesthetics ultimately before the experiments started.

7.3 Test units

Stainless steel chambers (width 10 cm, height 8.5 cm, depth 5.5 cm) served as test cages. The front side of the cages was closed with removable glass sheets; the bottom is perforated with ventilation holes (ø 1 mm). The top contains two openings for the food tube and entry for the bees. The inner sides of the cages (except front side) were covered with filter paper (Co. Machery & Nagel, D-52355 Düren, Art.No. 68). These test cages allowed an adequate rearing of the bees during the experiment combined with an easy observation through the front glass slide.

7.4 Test conditions

The test cages were exposed in incubators at 27 - 29 °C and 45 - 80 % relative humidity and darkness instead of 25 ± 2 °C and 60 -70 % relative humidity as mentioned in the EPPO guideline No. 170.
It can be excluded that this change had an influence on the results of this study (see 7.10 Deviation to the protocol).
Temperature and relative humidity were continuously recorded with a Thermo-Hygrometer.
The incubators were ventilated to avoid possible accumulations of pesticide vapour.

7.5 Food

During the tests, the bees were provided *ad libitum* with commercial ready to use syrup for honey bees as food (Apiinvert, Co. Südzucker AG, D-97199 Ochsenfurt).

7.6 Application of the test substance and of the controls

Each test was performed in five dosages (acute contact and oral toxicity test) of Confidor WG 70, one solvent control, one untreated negative control and one positive control with toxic standard (Dimethoate) with three replicates per dosage / control. One replicate was formed by one test cage containing 10 honey bees.

7.7 Course of the test

7.7.1 Contact toxicity test

Five dosages of the test substance were tested in order to provide a rational base for a proper assessment of the contact LD_{50} of Confidor WG 70 to honey bees.

Five solutions of the test substance in acetone/water (=solvent; 1:1 v/v) were prepared in adequate concentrations to yield the corresponding amounts per bee when 5 µl of the solution per individual were applied. The stock solution was prepared using 20 mg test substance suspended in 100 ml solvent (highest dosage group 0.2 µg/µl corresponding to 1 µg/bee/5 µl). This stock solution was diluted to 0.002 µg/µl in four steps. The following dosages were tested: 1; 0.5; 0.1, 0.05 and 0.01µg/bee.

For the negative control the bees only were anaesthetisized. For the solvent-control 5 µl/bee of the pure solvent was used.

For the positive control 0.2 µg/bee dimethoate in 5 µl solvent was used.

Immediately after preparing the test substance dilutions the application was done. Ultimately before applying the test substance the bees were anaesthetisized with CO_2. The CO_2 was given for 20 seconds directly into the test cages using one of the top openings. The anaesthetisized bees were laid, ventral surface up, on filter paper in petri dishes. One 5.0 µl drop per bee of Confidor WG 70 in solvent was placed on the ventral thorax using a Burkard-Applicator. The treated bees were then returned into the test cages and kept under test conditions for 48 hours. The duration of the anaesthetization was between 130 and 380 seconds until the total recovery of all individuals.

7.7.2 Oral toxicity test

Five dosages of the test substance, one control in water (solvent/ready to use syrup) and one positive control in ready to use syrup with dimethoate were tested in order to provide a rational base for a proper assessment of the oral LD_{50} of Confidor WG 70 to honey bees.

Five dilutions of the test substance in solvent/ready to use syrup (1 part solvent + 19 parts syrup) were prepared. 10 mg test substance was suspended in 200 ml water (= stock solution; corresponding to 50 µg/ml). 500 µl of this suspension was mixed with 9.5 ml Apiinvert (highest dosage group; ca. 0.05 µg/bee). The stock solution was diluted in 4 steps to yield the corresponding amounts per bee when ca. 30 mg of the dilution per individual were administered (0.01, 0.005, 0.001, 0.0005 µg/bee).

Test cages containing 10 bees each were prepared without food, letting the bees starve for 70 - 80 minutes. Within starving time of the bees preparation of the test substance dilutions was done. Following this, approximately 330 mg of the prepared dilutions in 1-ml syringes were hung into each cage through one of the top openings. The bees were observed as long as uptake of the dilution took place (uptake of contaminated food lasted not more than one hour). After the complete uptake of the test substance dilution the bees were provided with normal food (see 7.5).

The exact amount of test dilution consumed was determined by reweighing the syringes.

According to the weight results the following exact dosages were tested (details are given in table 5):

- 0.085 µg / bee
- 0.017 µg / bee
- 0.0088 µg / bee
- 0.0017 µg / bee
- 0.0009 µg / bee
- solvent/pure ready to use syrup control
- positive control (0.33 µg dimethoate / bee)

7.8 Test parameter

The experiments were started in the early midday to ensure a long enough observation time during the first day. The experiments were finished after 48 hours. Observation of the bees was continuous during the first 30 minutes after application of the test substance and then took place after the following times:

- 45 minutes, 60 minutes, 2 hours, 4 hours (first day)
- 24 hours, 48 hours (following days).

Any mortality and / or poisoning or behavioural abnormalities of the bees (e.g. food refuse, apathy, moving coordination problems) were recorded.

7.9 Evaluation of the results

Toxicity assessment of the test substance took place as comparison of the results obtained from the bees treated with test substance to those results obtained from the controls.

The study is valid because the mortality of the bees in the controls did not exceed 10 % within 24 hours and 48 hours.

The contact and oral LD_{50} (24 h and 48 h) of the test substance was estimated with probit analysis according to Finney 1971.

7.10 Deviations from the protocol

<u>Test conditions:</u>

Because of technical reasons the test containers were exposed at 48 - 85 % rel. humidity, instead of 40 - 70 % rel. humidity as described in the protocol and as mentioned in the EPPO guideline No. 170 (60 - 70 %).

Contact Toxicity Test:

The solvent was placed on the ventral thorax using a Burkard - Applicator, instead of a GC - syringe as described in the protocol, because using a Burkard - Applicator leads to an improvement of the study.

Because of technical reasons in five cages the duration of anaesthetization was between 320 and 380 seconds as indicated in the protocol (shorter than five minutes).

These deviations had no influence on the validity of the study.

8. Results and discussion

8.1 Contact toxicity test

Exposure of the honey bees to Confidor WG 70 caused behavioural abnormalities and mortality of the bees in all treatment groups. Mortality was dose-related and increased with increasing doses of the test substance (table 1; appendix table 3). The maximum and minimum mortality found after 48 hours of exposure was 76.7 % (1 µg/bee) and 0 % (0.01 µg/bee) respectively.

Most of the bees in the higher dosage groups (1 - 0.1 µg/bee) showed behavioural abnormalities (moving coordination problems, vomit, apathy and trembling) and were hyperactive compared to the untreated and solvent control groups.

96.7 % of the bees in the positive control group died until the end of the experiment after application of the toxic standard compound dimethoate.

One bee died in the solvent control and in the untreated negative controls two bees (6.7 %) died within the entire experimental time.

According to these results, the contact LD_{50} (24 h) of Confidor WG 70 to honey bees was calculated to be > 1 µg/ bee and the acute LD_{50} (48 h) was calculated to be 0.35 µg/bee (confidence range 0.25 to 0.51 µg/bee; probit analysis).

Table 1: Mortality and behavioural abnormalities of the bees in the contact toxicity test; presented results are averages from three replicates (10 bees each) per dosage/control.

See appendix, table 3 for details.

test article	first 30 minutes		after 1 hour		after 4 hours		after 24 hours		after 48 hours	
dosage µg/bee	mortality average %	behav. abnorm. average %	mortality average %	behav. abnorm. average %	mortality average %	behav. abnorm. average %	mortality average %	behav. abnorm. average %	mortality average %	behav. abnorm. average %
1.0	0.0	80.0	0.0	93.3	0.0	100.0	30.0	70.0	76.7	23.3
0.5	0.0	60.0	0.0	86.7	0.0	96.7	13.3	86.7	60.0	23.3
0.1	0.0	6.7	0.0	13.3	3.3	23.3	20.0	0.0	23.3	0.0
0.05	0.0	0.0	0.0	13.3	0.0	26.7	3.3	0.0	3.3	0.0
0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
untr.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7	0.0
solv.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.0
posit.	3.3	0.0	3.3	0.0	53.3	46.7	96.7	3.3	96.7	0.0

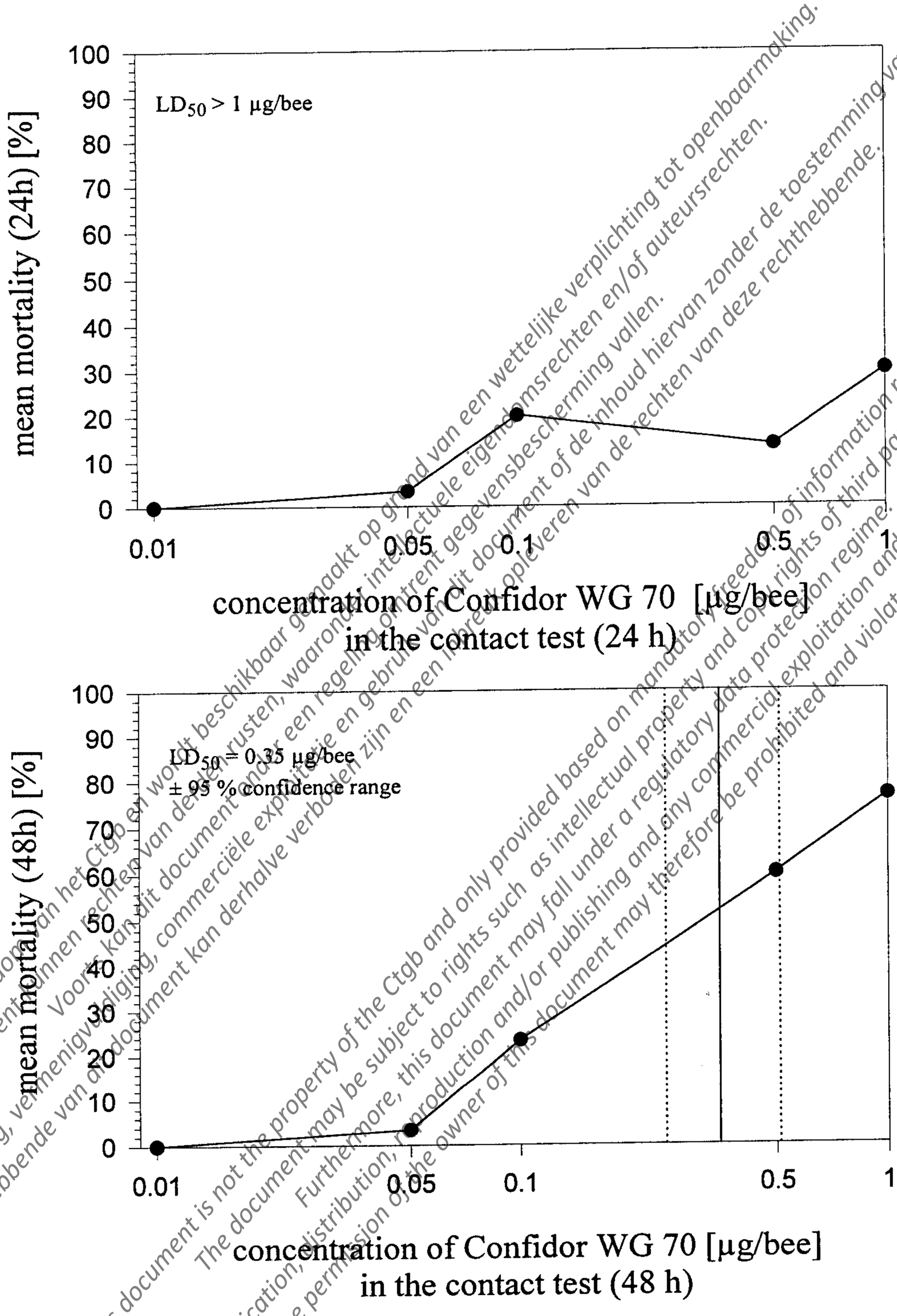


Fig. 1: Mortality of the honey bees in course of the contact test; above: mortality within 24 hours; below: mortality and LD_{50} within 48 hours;

8.2 Oral toxicity test

The amount of contaminated food ingested by the bees ranged from 29 to 40 mg/bee. According to the food uptake, the average dosages were calculated to 0.085, 0.017, 0.0088, 0.0017 and 0.0009 µg/bee (compare appendix table 5).

Ingestion of 0.085 µg/bee Confidor WG 70 resulted in 76.7 % mortality within 48 hours. The minimum mortality was 6.7 % (0.0017 µg/bee). No mortality was observed after the ingestion of 0.0009 µg/bee.

100 % of the bees in the positive control group died within the experimental time (48 h). In the negative control group one bee (3.3 %) died within the whole experimental time.

The results of this experiment give a dose-effected relationship.

The mortality after 48 hours was 76.7 %, 56.7 %, 40.0 and 6.7 % in the 0.085, 0.017, 0.0088 and 0.0017 µg/bee treatment groups.

According to these data, the acute oral LD_{50} (24 h) was calculated to be ≥ 0.085 µg/bee and the acute oral LD_{50} (48 h) was calculated to be 0.0167 µg/bee (95 % confidence range from 0.0105 to 0.0264 µg/bee; probit analysis).

Table 2: Mortality and behavioural abnormalities of the bees in the oral toxicity test; presented results are averages from three replicates (10 bees each) per dosage/control. negative = negative control, positive = positive control (treated with dimethoate). See appendix, table 4 for details.

uptaken test substance µg/bee	first 30 minutes mortality average %	first 30 minutes behav. abnorm. average %	after 1 hour mortality average %	after 1 hour behav. abnorm. average %	after 4 hours mortality average %	after 4 hours behav. abnorm. average %	after 24 hours mortality average %	after 24 hours behav. abnorm. average %	after 48 hours mortality average %	after 48 hours behav. abnorm. average %
0.085	0.0	0.0	0.0	100.0	0.0	100.0	36.7	40.0	76.7	0.0
0.017	0.0	0.0	0.0	0.0	10.0	63.3	53.3	13.3	56.7	0.0
0.0088	0.0	0.0	0.0	0.0	10.0	36.7	40.0	0.0	40.0	0.0
0.0017	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7	0.0
0.0009	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
negative	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.0
positive	3.3	0.0	6.7	0.0	40.0	40.0	96.7	13.3	100.0	0.0

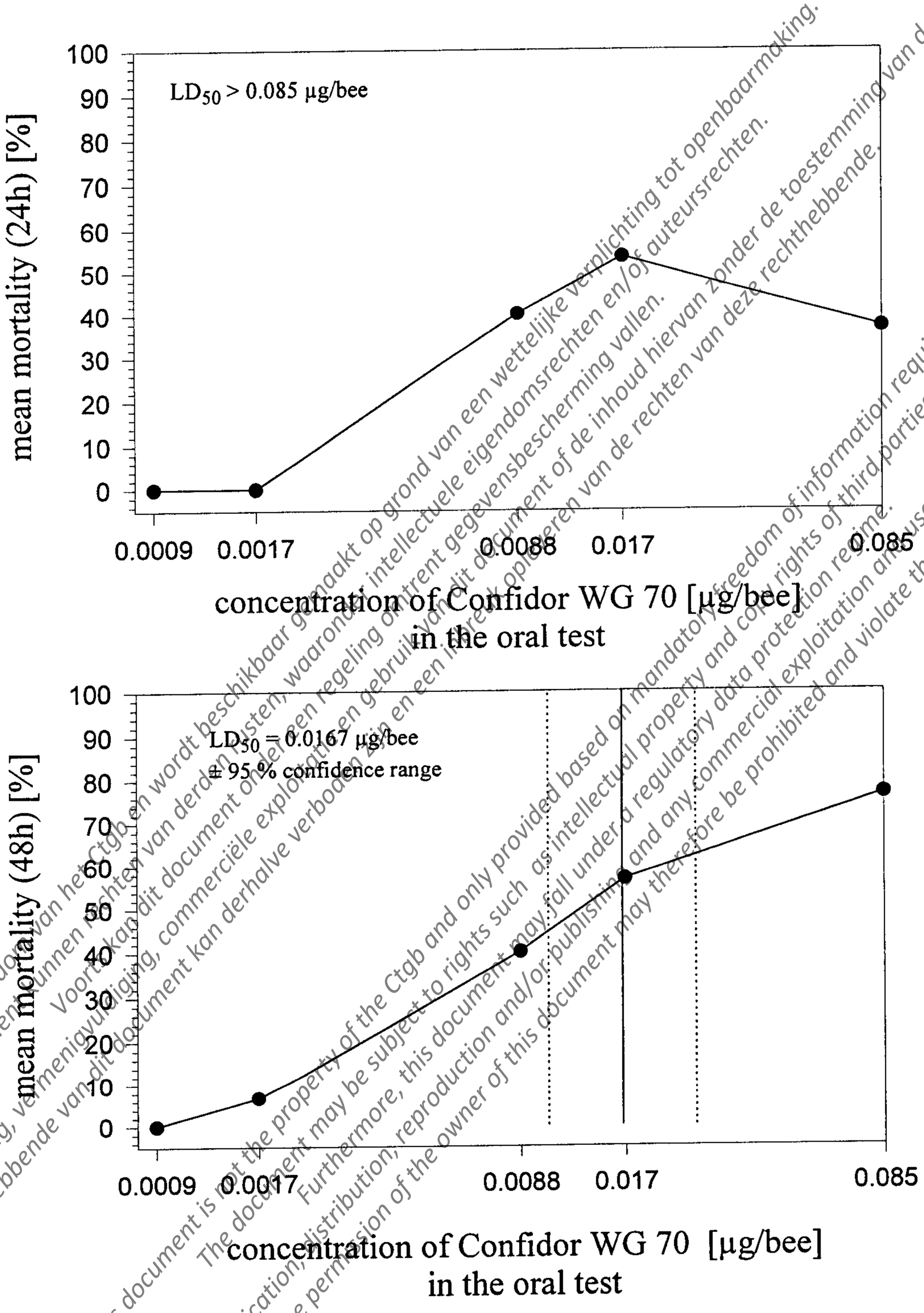


Fig. 2: Mortality of the honey bees in course of the oral test; above: mortality within 24 hours; below: mortality and LD_{50} within 48 hours;

8.3 Conclusions

The results of this study exhibit effects of Confidor WG 70 to honey bees in the contact and oral toxicity test. The contact LD_{50} of Confidor WG 70 was calculated to be 0.35 µg/bee (48 h) and > 1 µg/bee (24 h) respectively.

The oral LD_{50} of Confidor WG 70 was calculated to be ≥ 0.085 µg/bee (24 h) and 0.0167 µg/bee (48 h) respectively.

9. Literature

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

Finney D.J. 1971: Probit Analysis. 3rd Edition, Cambridge University Press, London

10. Distribution of the report

Sponsor: 2x (original and one copy)

Study director: 1x (copy)

Appendix

Table 3: definitive contact toxicity test; mortality and behavioural abnormalities of the bees under the influence of several dosages of the test substance;

Observations: mortality and behavioural abnormalities; # = number of individuals; sympt. = observed symptoms according to the following key:

1 = vomit; 2 = moving coordination problems; 3 = apathy; 4 = intensive cleaning 5 = trembling

vial	test article	first 30 minutes			after 45 minutes			after 1 hour			after 2 hours			after 4 hours			after 24 hours			after 48 hours		
	dosage	dead	behav.abnor.		dead	behav.abnor.		dead	behav.abnor.		dead	behav.abnor.		dead	behav.abnor.		dead	behav.abnor.		dead	behav.abnor.	
#	µg/bee	#	#	sympt	#	#	sympt	#	#	sympt	#	#	sympt	#	#	sympt	#	#	sympt	#	#	sympt
1	1.0	0	8	1,3	0	8	1,3	0	8	2,3,5	0	10	3	0	10	2,3	2	8	1,2,3	7	3	2,3
2	1.0	0	6	1,3	0	10	1,2,3	0	10	2,3,5	0	10	2,3,5	0	10	2,3	3	7	1,2,3	8	2	2
3	1.0	0	10	1,3	0	10	1,3	0	10	2,3,5	0	10	2,3,5	0	10	2,3	4	6	1,2,3	8	2	2
1	0.5	0	6	1,2	0	6	1,3	0	10	1,2,3	0	10	2,3	0	10	2,3	3	7	2	10	0	
2	0.5	0	5	1,4	0	9	1,3	0	10	1,2,3	0	10	2,3,5	0	10	2,3	0	10	2,3	5	5	2,3
3	0.5	0	7	1,4	0	9	1,3	0	6	3	0	10	2,3,5	0	9	2,3	1	9	2,3	3	2	2,3
1	0.1	0	1	2	0	0		0	1	2	0	6	2,3	1	2	2,3	2	0		3	0	
2	0.1	0	1	2	0	1	3	0	2	3	0	4	2,3	0	3	3	2	0		2	0	
3	0.1	0	0		0	0		0	1	3	0	3	3	0	2	2	2	0		2	0	
1	0.05	0	0		0	1	2	0	0		0	1	3	0	4	3	0	0		0	0	
2	0.05	0	0		0	1	2	0	2	4	0	1	4	0	2	3	0	0		0	0	
3	0.05	0	0		0	0		0	2	4	0	2	3	0	2	3	1	0		1	0	
1	0.01	0	0		0	0		0	0		0	0		0	0		0	0		0	0	
2	0.01	0	0		0	0		0	0		0	0		0	0		0	0		0	0	
3	0.01	0	0		0	0		0	0		0	0		0	0		0	0		0	0	
1	untreated control	0	0		0	0		0	0		0	0		0	0		0	0		0	0	
2	control	0	0		0	0		0	0		0	0		0	0		0	0		1	0	
3	control	0	0		0	0		0	0		0	0		0	0		0	0		1	0	
1	solvent control	0	0		0	0		0	0		0	0		0	0		0	0		0	0	
2	control	0	0		0	0		0	0		0	0		0	0		0	0		1	0	
3	control	0	0		0	0		0	0		0	0		0	0		0	0		0	0	
1	positive control	1	0		1	0		1	0		1	9	2,3	5	5	2,3	10	0		10	0	
2	control	0	0		0	0		0	0		1	9	2,3	4	6	2,3	9	1	2	9	0	
3	control	0	0		0	0		0	0		0	8	2,3	7	3	2,3	10	0		10	0	

Table 3 (continued): definitive contact toxicity test; mortality and behavioural abnormalities of the bees
average ± standard deviation from four replicates per concentration / control.

avg. ± sd = average ± standard deviation; behav. abnorm. = behavioural abnormalities;

test article dosage	first 30 minutes				after 1 hour				after 4 hours				after 24 hours				after 48 hours			
	mortality		behav.abnor.		mortality		behav.abnor.		mortality		behav.abnor.		mortality		behav.abnor.		mortality		behav.abnor.	
	avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd	
µg/bee	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
1.0	0.0	0.0	80.0	20.0	0.0	0.0	93.3	11.5	0.0	0.0	100.0	0.0	30.0	10.0	70.0	10.0	76.7	5.8	23.3	5.8
0.5	0.0	0.0	60.0	10.0	0.0	0.0	86.7	23.1	0.0	0.0	96.7	5.8	13.3	15.3	86.7	15.3	60.0	36.1	23.3	25.2
0.1	0.0	0.0	6.7	5.8	0.0	0.0	13.3	5.8	3.3	5.8	23.3	5.8	20.0	0.0	0.0	0.0	23.3	5.8	0.0	0.0
0.05	0.0	0.0	0.0	0.0	0.0	0.0	13.3	11.5	0.0	0.0	26.7	11.5	3.3	5.8	0.0	0.0	3.3	5.8	0.0	0.0
0.01	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	0.0	0.0	0.0	0.0
untr.	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	6.7	5.8	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	3.3	5.8	0.0	0.0
posit.	3.3	5.8	0.0	0.0	3.3	5.8	0.0	5.8	53.3	15.3	46.7	15.3	96.7	5.8	3.3	5.8	96.7	5.8	0.0	0.0

Table 4: definitive oral toxicity test; mortality and behavioural abnormalities of the bees under the influence of several dosages of the test substance; test article dosages calculated after reweighing the syringes.

Observations: mortality and behavioural abnormalities; # = number of individuals; sympt. = observed symptoms according to the following key;
1 = food refuse/vomit; 2 = moving coordination problems; 3 = apathy; 4 = intensive cleaning 5 = trembling

vial	test article	first 30 minutes			after 45 minutes			after 1 hour			after 2 hours			after 4 hours			after 24 hours			after 48 hours		
#	dosage µg/bee	dead #	behav. #	abnor sympt	dead #	behav. #	abnor sympt	dead #	behav. #	abnor sympt	dead #	behav. #	abnor sympt	dead #	behav. #	abnor sympt	dead #	behav. #	abnor sympt	dead #	behav. #	abnor sympt
1	0.096	0	0		0	6	3,4,5	0	10	3	0	10	3	0	10	2,3	3	7	2,3	8	0	
2	0.073	0	0		0	10	2,3	0	10	2,3	0	9	3	0	10	2,3	4	3	2	8	0	
3	0.085	0	0		0	10	2	0	10	2,3	0	10	3	0	10	2,3	4	2	2	7	0	
1	0.017	0	0		0	0		0	0		0	1	2	1	9	2,3	4	4	2	5	0	
2	0.017	0	0		0	0		0	0		0	1	2	1	5	2,3	7	0		7	0	
3	0.017	0	0		0	0		0	0		0	0		1	5	2,3	5	0		5	0	
1	0.010	0	0		0	0		0	0		0	1	2	2	5	2	4	0		4	0	
2	0.009	0	0		0	0		0	0		0	0		1	3	2	5	0		5	0	
3	0.008	0	0		0	0		0	0		0	0		0	3	2	3	0		3	0	
1	0.0017	0	0		0	0		0	0		0	0		0	0		0	0		0	0	
2	0.0017	0	0		0	0		0	0		0	0		0	0		0	0		0	0	
3	0.0017	0	0		0	0		0	0		0	0		0	0		0	0		2	0	
1	0.0008	0	0		0	0		0	0		0	0		0	0		0	0		0	0	
2	0.0009	0	1	2	0	0		0	0		0	0		0	0		0	0		0	0	
3	0.0009	0	0		0	0		0	0		0	0		0	0		0	0		0	0	
1	negative control	0	0		0	0		0	0		0	0		0	0		0	0		0	0	
2	control	0	0		0	0		0	0		0	0		0	0		0	0		0	0	
3	control	0	0		0	0		0	0		0	0		0	0		0	0		1	0	
1	positive control	0	0		0	0		0	0		1	2	2	2	6	2,3	10	0		10	0	
2	control	1	0		1	1	3	2	0		3	5	2,3	5	2	3	9	1	3	10	0	
4	control	0	0		0	0		0	0		3	3	2	5	4	3	10	3	3	10	0	

Table 4 (continued): definitive oral toxicity test; mortality and behavioural abnormalities of the bees;

average ± standard deviation from three replicates per concentration / control.

avg. ± sd = average ± standard deviation; behav. abnorm. = behavioural abnormalities;

test article dosage avg. µg/bee	first 30 minutes				after 1 hour				after 4 hours				after 24 hours				after 48 hours			
	mortality		behav.abnor.		mortality		behav.abnor.		mortality		behav.abnor.		mortality		behav.abnor.		mortality		behav.abnor.	
	avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd		avg. ± sd	
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
0.085	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	100.0	0.0	36.7	5.8	40.0	26.5	76.7	5.8	0.0	0.0
0.017	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	63.3	23.1	53.3	15.3	13.3	23.1	56.7	11.5	0.0	0.0
0.0088	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	10.0	36.7	11.5	40.0	10.0	0.0	0.0	40.0	10.0	0.0	0.0
0.0017	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	6.7	11.5	0.0	0.0
0.0009	0.0	0.0	3.3	5.8	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
negative	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	3.3	5.8	0.0	0.0
positive	3.3	5.8	0.0	0.0	6.7	11.5	0.0	0.0	40.0	17.3	40.0	20.0	96.7	5.8	13.3	5.8	100.0	0.0	0.0	0.0

Table 5: definitive oral intake test: weight of syringes at start of the experiment and after removing from the test cages; uptaken solution as calculated average (difference/bee)

test substance dosage nominal	test substance concentrations in food µg / mg	weight of syringes start mg	weight of syringes end mg	difference mg	uptaken solution mg / bee	uptaken test substance µg / bee	uptaken test substance ø µg / bee
0.05 µg/bee							
1	0.0025	3521	3136	385	39	0.096	
2	0.0025	3638	3345	293	29	0.073	0.085
3	0.0025	3566	3228	338	34	0.085	
0.01 µg/bee							
1	0.0005	3645	3306	339	34	0.017	
2	0.0005	3560	3221	339	34	0.017	0.017
3	0.0005	3503	3168	335	34	0.017	
0.005 µg / bee							
1	0.00025	3700	3304	396	40	0.0099	
2	0.00025	3500	3136	364	36	0.0091	0.0088
3	0.00025	3529	3228	301	30	0.0075	
0.001 µg / bee							
1	0.00005	3621	3280	341	34	0.0017	
2	0.00005	3614	3278	336	34	0.0017	0.0017
3	0.00005	3690	3341	349	35	0.0017	
0.0005 µg / bee							
1	0.000025	3550	3219	331	33	0.0008	
2	0.000025	3482	3129	353	35	0.0009	0.0009
3	0.000025	3723	3376	347	35	0.0009	
negative control							
1	0	3587	3287	300	30	0.000	
2	0	3545	3238	307	31	0.000	0.0
3	0	3515	3183	332	33	0.000	
positive control							
Dimethoate	µg/mg					µg/bee	µg/bee
0.2 µg/bee							
1	0.01	3620	3273	347	35	0.347	
2	0.01	3479	3148	331	33	0.331	0.33
3	0.01	3383	3065	318	32	0.318	