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Final Report

Laboratory Testing for Toxicity (Acute Oral LD_{50}) of NTN 33893 on Honey Bees (*Apis mellifera* L.) (Hymenoptera, Apidae)

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

[redacted]

Study Completion Date: September 30, 1999

Sponsor

Bayer AG
Crop Protection Division
Institute for Environmental Biology
Alfred-Nobel-Str. 50
40789 Monheim
Germany

Test Facility

Institut für Biologische Analytik
und Consulting IBACON GmbH
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64380 Rossdorf
Germany

Project 6400036

Copy of GLP Certificate

[redacted]

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List of Tables

1. Summary

Report: [redacted] (1999): Laboratory Testing for Toxicity (Acute Oral LD_{50}) of NTN 33893 on Honey Bees (*Apis mellifera* L.) (Hymenoptera, Apidae).
Source: IBACON, unpublished report No.: 6400036, September 30, 1999.

Guidelines: EPPO No. 170
Deviations: temperature: 29 °C; relative humidity: 50 - 70 % 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: NTN 33893, purity: 99.4%, batch number: M00680; under laboratory conditions, starved honey bees (*Apis mellifera*, 3 groups of 10 bees per dose) received a single oral dose of either 40.9, 22.9, 12.2, 6.0, 3.1, 1.5, 0.8 or 0.1 ng per bee in ca. 20 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. 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	NTN 33893
Test object	*Apis mellifera*
Application rates ng product/bee	40.9*, 22.9*, 12.2*, 6.0*, 3.1*, 1.5*, 0.8* and 0.1*
Exposure	oral (sugar solution)
LD_{50} ng product/bee (48 and 96h)	approximately 40.9

* values based on actual intake of the test substance

Observations: the observation period was extended for 48 hours because of delayed mortality in the higher dose groups. No treatment-related mortalities or behavioural impacts were recorded at oral doses of 1.5 ng/bee and lower. Oral doses of 3.1 ng/bee and higher caused treatment-related mortalities and behavioural impacts such as apathy and exaggerated/discoordinated movements. The behavioural impacts lasted dose-related up to 48 hours. In the control, three of 30 bees (3.3%) died whereas all bees died in the groups treated with the toxic standard.

2. Survey of the Study

2.1 General Information

Title:	Laboratory Testing for Toxicity (Acute Oral LD_{50}) of NTN 33893 on Honey Bees (*Apis mellifera* L.) (Hymenoptera, Apidae)
Sponsor:	Bayer AG Crop Protection Division Institute for Environmental Biology Alfred-Nobel-Str. 50 40789 Monheim Germany
Monitoring:	[redacted]
Test Substance:	NTN 33893
Test Facility:	Institut für Biologische Analytik und Consulting IBACON GmbH Industriestrasse 1 64380 Rossdorf Germany
IBACON Project:	6400036
Project Staff:	
Test Facility Management:	[redacted]
Study Director:	
Technical Coordination:	
Head of Quality Assurance Unit (QAU):	
Quality Assurance Unit Manager:	
Schedule:	
Study Initiation Date:	June 14, 1999
Date of 1st Amendment to Study Protocol:	June 24, 1999
Date of 2nd Amendment to Study Protocol:	August 25, 1999
Date of 3rd Amendment to Study Protocol:	September 29, 1999
Experimental Starting Date:	July 6, 1999
Experimental Completion Date:	July 10, 1999
Draft Report Date:	September 29, 1999
Study Completion Date:	September 30, 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
- all study protocol amendments
- 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
Industriestrasse 1
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: [redacted]

date: September 30, 1999

Test Facility Management: [redacted]

date: September 30, 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 NTN 33893 on Honey Bees (*Apis mellifera* L.) (Hymenoptera, Apidae)

5.2 Purpose

If honey bees can be exposed to residues of NTN 33893, 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:

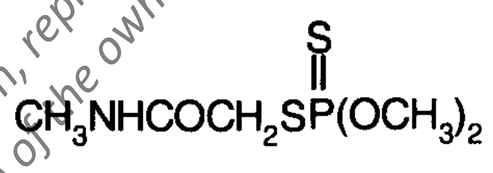
Name:	NTN 33893
Batch No.: (Lot No.)	M00680
Active Ingredient(s)/Purity:	99.4 % according to certificate of analysis (certificate available by the sponsor)
Certificate of Analysis Ref. Code / Date:	March 13, 1998
Indication:	insecticide
Aggregate State at Room Temperature:	solid
Colour:	white (according to IBACON personnel)
Solubility:	in water: ca 0.5 g/L
Stability:	pure: see expiry date in water: test substance must be considered as stable under test conditions
Expiry Date:	March 2000
Storage:	in original container, 0 - 10 °C, in the dark

Control

Oral Test:	tap water

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:	29 °C[1]
Relative Humidity:	50 - 70 %[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:	*ca.* 20 mg of NTN 33893 contaminated food (1 part solvent + 19 parts syrup) and offered in syringes which were weighed before and after introduction into the cages (duration of uptake did not exceed 3 hours)
Dosages of the Test Substance in the Oral Test:	40.9, 22.9, 12.2, 6.0, 3.1, 1.5, 0.8 and 0.1 ng/bee (values based on actual intake of the test substance)
Dosage of the Toxic Standard:	0.2 µg a.i. Dimethoate per bee (oral test)
Control:	NTN 33893 free sugar solution

6.7 Course of the Test

Treatment Groups:	control, 8 dosages of test substance, toxic standard
Replicates:	3 per treatment group
Individuals:	10 per unit, 30 individuals per treatment group
Starvation Time:	70 minutes
Exposure Time:	96 hours (because of increasing mortality between 24 and 48 hours the test duration was prolonged)

6.8 Test Parameters

Mortality:	number of dead bees after 60 minutes; 2, 4 hours (first day); 24 and 48 hours and additionally 72 and 96 hours
Behavioural Abnormalities:	behavioural abnormalities (vomiting, apathy, intensive cleaning, discoordinated movements) after 60 minutes; 2, 4 hours (first day); 24 and 48 hours and additionally 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:	10 % at experimental end (96 hours)
Toxic Standard Mortality:	resulted in 100 % mortality

6.11 Deviations to the Study Protocol

Concerning:	Storage of the test substance
According to the Study Protocol:	0 - 10 °C
Deviation to the Study Protocol:	test substance was stored in a refrigerator at room temperature, with a minimum temperature of -1.1 °C instead of 0 - 10 °C as indicated in the study protocol
Reason for the Deviation:	because of technical reasons the temperature had a broader range than expected
Presumed Effect on the Study:	none, because the measured temperature range is a minimum and maximum range which appears only for a short time interval

Concerning:	course of the test; individuals
According to the Study Protocol:	10 individuals per replicate and treatment group
Deviation to the Study Protocol:	one bee escaped during the experimental time in the 1.5 ng test substance group.
Reason for the Deviation:	technical reason.
Presumed Effect on the Study:	the mortality data were calculated taking into consideration 29 bees instead of 30 after the escape of the bee.

7. Results and Discussion

7.1 Oral Toxicity Test

Because of increasing mortality between 24 and 48 hours, the experiment was prolonged for further 48 hours up to 96 hours. After a starving period of 70 minutes, mortality occurred after ingestion of 40.9 (53.3 %), 22.9 (16.7 %) , 12.2 (30.0 %), 6.0 (33.3 %), 3.1 (33.3 %), and 1.5 (6.9 %) ng NTN 33893 per bee at the end of the experiment. No mortality occurred in the 0.8 and 0.1 ng NTN 33893 treated groups. Behavioural abnormalities like apathy, laziness and nervousness were observed in the 40.9, 22.9, 12.2, 6.0 and 3.1 ng NTN 33893 treated groups during the first 48 hours of the experiment. No further behavioural abnormalities occurred. After ingestion of 1.5, 0.8 and 0.1 ng NTN 33893 no behavioural abnormalities were observed (Table 1, Appendix Table 2 - 3).

The oral LD_{50} of NTN 33893 must be estimated as approximately 40.9 ng/bee.

In the controls three of the 30 bees (10 %) bee died 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 ng/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 %
40.9	6.7	86.7	6.7	93.3	26.7	30.0	50.0	3.3	53.3	0.0	53.3	0.0
22.9	3.3	30.0	3.3	93.3	13.3	16.7	16.7	0.0	16.7	0.0	16.7	0.0
12.2	3.3	0.0	3.3	96.7	23.3	10.0	30.0	0.0	30.0	0.0	30.0	0.0
6.0	0.0	0.0	6.7	40.0	33.3	0.0	33.3	0.0	33.3	0.0	33.3	0.0
3.1	0.0	0.0	10.0	16.7	33.3	0.0	33.3	0.0	33.3	0.0	33.3	0.0
1.5	0.0	0.0	3.4	0.0	3.4	0.0	3.4	0.0	3.4	0.0	6.9	0.0
0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1	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	6.7	3.3	3.3	6.7	0.0	10.0	0.0	10.0	0.0	10.0	0.0
toxic st.	0.0	0.0	80.0	13.3	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; solv. = solvent control; toxic st. = toxic standard

7.2 Conclusions

Mortality:	Oral $LD_{50\ (48\ and\ 96h)}$ of NTN 33893: approximately 40.9 ng/bee
Behavioural Abnormalities:	During the first 48 hours e.g. apathy and nervousness occurred in all dosage groups, except after ingestion of 1.5, 0.8 and 0.1 ng test substance per bee.

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 ng/bee	dead #	beh. #	abnor. symp.	dead #	beh. #	abnor. symp.	dead #	beh. #	abnor. symp.	dead #	beh. #	abnor. symp.	dead #	beh. #	abnor. symp.
1	46.2	0	10	c	0	9	b,c	0	10	c	1	4	b	8	0	
2	31.0	1	7	c,e	1	9	c	1	9	c	4	3	b,c	4	1	e
3	45.4	1	9	c	1	9	c	1	9	c	3	2	b,c	3	0	
1	23.8	0	6	c,f	0	10	c,f	0	9	c	0	1	c	0	0	
2	21.2	1	0		1	6	c	1	9	c	1	1	b	1	0	
3	23.7	0	3	b,e	0	9	c	0	10	c,e,b	3	3	c	4	0	
1	12.1	0	0		0	10	c,b	0	10	c	0	2	c	1	0	
2	12.1	1	0		1	7	c	1	9	b,c,e	4	1	c	5	0	
3	12.4	0	0		0	8	c	0	10	c,e	3	0		3	0	
1	5.8	0	0		0	0		1	0		2	0		2	0	
2	6.1	0	0		0	8	b,c	1	9	c	4	0		4	0	
3	6.1	0	0		0	1	b	0	3	c	4	0		4	0	
1	3.0	0	0		0	1	e	1	4	c,e	5	0		5	0	
2	3.1	0	0		0	0		1	1	b	4	0		4	0	
3	3.1	0	0		0	1	b	1	0		1	0		1	0	
1	1.5	0	0	*	0	0		1	0		1	0		1	0	
2	1.6	0	0		0	0		0	0		0	0		0	0	
3	1.5	0	0		0	0		0	0		0	0		0	0	
1	0.8	0	0		0	0		0	0		0	0		0	0	
2	0.8	0	0		0	0		0	0		0	0		0	0	
3	0.8	0	0		0	0		0	0		0	0		0	0	
1	0.1	0	0		0	0		0	0		0	0		0	0	
2	0.1	0	0		0	0		0	0		0	0		0	0	
3	0.1	0	0		0	0		0	0		0	0		0	0	
1	solvent control	0	2	c	1	1	c	1	1	c	2	0		3	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	
1	toxic standard	0	0		0	1	b	10	0		10	0		10	0	
2	standard	0	0		1	0		7	3	c	10	0		10	0	
3	standard	0	0		2	3	b	7	1	c	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;

* one bee escaped from the test unit; c = apathy; d= intensive cleaning; e=nervous; f = sitting in one corner of the chamber

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] ng/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	46.2	8	0		8	0	
2	31.0	5	0		5	0	
3	45.4	3	0		3	0	
1	23.8	0	0		0	0	
2	21.2	1	0		1	0	
3	23.7	4	0		4	0	
1	12.1	1	0		1	0	
2	12.1	5	0		5	0	
3	12.4	3	0		3	0	
1	5.8	2	0		2	0	
2	6.1	4	0		4	0	
3	6.1	4	0		4	0	
1	3.0	5	0		5	0	
2	3.1	4	0		4	0	
3	3.1	1	0		1	0	
1	1.5	1	0		1	0	
2	1.6	0	0		0	0	
3	1.5	0	0		1	0	
1	0.8	0	0		0	0	
2	0.8	0	0		0	0	
3	0.8	0	0		0	0	
1	0.1	0	0		0	0	
2	0.1	0	0		0	0	
3	0.1	0	0		0	0	
	solvent						
1	control	3	0		3	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

Table 3. (Relative Data, continued). 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]	
	mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD
ng/bee	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
40.9	6.7	5.8	86.7	15.3	6.7	5.8	93.3	5.8	26.7	15.3	30.0	10.0	50.0	26.5	3.3	5.8
22.9	3.3	5.8	30.0	30.0	3.3	5.8	93.3	5.8	13.3	15.3	16.7	11.5	16.7	20.8	0.0	0.0
12.2	3.3	5.8	0.0	0.0	3.3	5.8	96.7	5.8	23.3	20.8	10.0	10.0	30.0	20.0	0.0	0.0
6.0	0.0	0.0	0.0	0.0	6.7	5.8	40.0	45.8	33.3	11.5	0.0	0.0	33.3	11.5	0.0	0.0
3.1	0.0	0.0	0.0	0.0	10.0	0.0	16.7	20.8	33.3	20.8	0.0	0.0	33.3	20.8	0.0	0.0
1.5	0.0	0.0	0.0	0.0	3.4	6.4	0.0	0.0	3.4	6.4	0.0	0.0	3.4	6.4	0.0	0.0
0.8	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
0.1	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	6.7	11.5	3.3	5.8	3.3	5.8	6.7	11.5	0.0	0.0	10.0	17.3	0.0	0.0
toxic st.	0.0	0.0	0.0	0.0	80.0	17.3	13.3	15.3	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

solv. = solvent treated 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]	
mean	mean	±SD	mean	±SD	mean	±SD	mean	±SD
ng/bee	%	%	%	%	%	%	%	%
40.9	53.3	25.2	0.0	0.0	53.3	25.2	0.0	0.0
22.9	16.7	20.8	0.0	0.0	16.7	20.8	0.0	0.0
12.2	30.0	20.0	0.0	0.0	30.0	20.0	0.0	0.0
6.0	33.3	11.5	0.0	0.0	33.3	11.5	0.0	0.0
3.1	33.3	20.8	0.0	0.0	33.3	20.8	0.0	0.0
1.5	3.4	6.4	0.0	0.0	6.9	6.1	0.0	0.0
0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
solvent	10.0	17.3	0.0	0.0	10.0	17.3	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;
solv. = solvent control; toxic st. = toxic standard
±SD = standard deviation from three replications

Table 4. Definitive oral intake

substance dosage nominal	substance concentrations in food ng / mg	weight of syringes start[a] mg	end[b] mg	difference mg	uptaken solution mg / bee	uptaken substance ng / bee[d]	ø[c] ng / bee
40 ng/bee							
1	2	11247	11016	231	23	46.2	
2	2	11252	11097	155	16	31.0	40.9
3	2	11276	11049	227	23	45.4	
20 ng/bee							
1	1	11173	10935	238	24	23.8	
2	1	11286	11074	212	21	21.2	22.9
3	1	11208	10971	237	24	23.7	
10 ng / bee							
1	0.5	11178	10936	242	24	12.1	
2	0.5	11250	11009	241	24	12.1	12.2
3	0.5	11249	11002	247	25	12.4	
5 ng / bee							
1	0.25	11225	10995	230	23	5.8	
2	0.25	11329	11086	243	24	6.1	6.0
3	0.25	11290	11045	245	25	6.1	
2.5 ng / bee							
1	0.125	11223	10980	243	24	3.0	
2	0.125	11241	10991	250	25	3.1	3.1
3	0.125	11213	10962	251	25	3.1	
1.3 ng / bee							
1	0.0625	11216	10972	244	24	1.5	
2	0.0625	11271	11021	250	25	1.6	1.5
3	0.0625	11122	10883	239	24	1.5	
0.6 ng / bee							
1	0.031	11201	10957	244	24	0.8	
2	0.031	11202	10944	258	26	0.8	0.8
3	0.031	11255	11007	248	25	0.8	
0.1 ng / bee							
1	0.005	11209	10958	251	25	0.1	
2	0.005	11211	10964	247	25	0.1	0.1
3	0.005	11222	10972	250	25	0.1	
solvent control							
1	0	11235	10982	253	25	0.0	
2	0	11213	10967	246	25	0.0	0.0
3	0	11204	10957	247	25	0.0	
toxic standard							
Dimethoate							
0.2 µg/bee							
1	0.01	11212	10968	244	24	0.2	
2	0.01	11248	11003	245	25	0.2	0.2
3	0.01	11238	10992	246	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 Solutions

Oral Test

Prior to the dilution process, the stock solution was a clear fluid.

20 mg of the test substance was dissolved ad 500 g tap water (stock solution). This was done one day before the application. The stock solution was stirred overnight in a refrigerator.

Six 1 :2 dilutions of the stock solution were done step by step.

The 0.1 ng / bee concentration was obtained by diluting the 10 ng/bee dilution 1 : 100.

500 mg of each 1:2 dilution, 500 mg of the 1:100 dilution of the 10 ng dose and 500 mg of the stock solution were added to 9.5 g syrup (1:20) for preparation of the 8 different treatment solutions (reference not included).

40, 20, 10, 5, 2.5, 1.3, 0.6 and 0.1 ng/bee should be obtained when 20 mg of the contaminated sugar solution per bee were administered.

40.9, 22.9, 12.2, 6.0, 3.1, 1.5, 0.8 and 0.1 ng/bee were obtained, because the bees ingested between 16 and 26 mg contaminated food per bee.