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Labor für biologische und chemische Analytik GmbH

FINAL REPORT

Substance C

STUDY TITLE

Acute oral toxicity of Substance C to the honeybee *Apis mellifera* L. under laboratory conditions prolonged for 10 days

Guidelines:

EPPO Standard PP 1/170(2) (1999); OECD 213 (1998)

Author

[redacted]

STUDY COMPLETED ON

July 04, 2000

PERFORMING LABORATORY

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LABORATORY PROJECT IDENTIFICATION

BioChem agrar project Number: 00 10 48 0502c

SPONSOR

Bayer AG
Landwirtschaftszentrum Monheim
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SUMMARY

Study title:	Acute toxicity of Substance C to the honeybee *Apis mellifera* L. under laboratory conditions
Guideline(s):	EPPO Standard PP 1/170(2) (1999); OECD 213 (1998)
BioChem project No.:	00 10 48 502c
Test substance:	Substance C
Formulation:	white powder
Test species:	*Apis mellifera carnica* L. stage: adults (field and house bees) source: purchased from bee keeper [redacted]
Test system:	oral toxicity test of Substance C on honeybees
Test conditions:	temperature: 24-26 °C relative humidity: 63-75 % lighting intensity: ca 100 lx photoperiod: constant darkness throughout the test (with the exception of diffuse artificial light for less than 15 hours (over the whole test period) for the treatment and observations (ca 100 lx))
Treatments:	control, 3 test substance concentrations
Test substance treatment levels:	the test substance was applied at the following rates: oral toxicity test: 10 ppb, 1 ppb and 0.1 ppb (equivalent to 10, 1.0 and 0.1 ng Substance C/g sucrose solution)
Replicates:	5 replicates per treatment, each with 10 bees
Exposure time:	10 days
Biological observations:	mortality: 4, 24, 48 hours and once daily up to 10 days

Results:

During a 10- day test period the bees consumed sucrose solution containing 0.1, 1 and 10 ppb Substance C. The amount of consumed sucrose solution was summed up for the whole test duration. The total amount of sucrose solution containing the test substance was used to determine the total amount of test substance consumed per bee. The test endpoints were mortality and behaviour of the honeybees in comparison with the control.

House bees:

No statistically significant effects on honeybee mortality were observed after oral exposure to Substance C at concentrations of 0.1, 1.0 and 10 ppb test substance per bee.
The test substance at concentrations of 0.1, 1.0 and 10.0 ppb Substance C per bee caused 10.0 %, 4 % and 6 % mortality after 10 days.
Therefore it is concluded that providing the test substance sucrose solution containing Substance C up to 10 ppb (equivalent to 8.056 ng test substance C consumed/bee) over the prolonged test duration of 10 days had no impact on bee mortality.
No effects on the behaviour of the bees (or other sublethal effects) were observed in comparison with the control bees.

Field bees

No statistically significant effects on honeybee mortality were observed after oral exposure to Substance C at concentrations of 0.1, 1.0 and 10.0 ppb Substance C per bee.
The test substance at concentrations of 0.1, 1.0 and 10 ppb Substance C per bee caused 30 %, 40 % and 32 % mortality after 10 days. The increasing mortality observed starting with day 7 was observed for all treatment groups including the control. The sensibility of field bees (including the control treatment) compared to house bees was significantly higher. Therefore a higher overall mortality was observed in the field bee oral toxicity test.

Therefore it is concluded that providing the test substance sucrose solution containing the Substance C up to 10 ppb (equivalent to 8.056 ng Substance C/bee) over the prolonged test duration of 10 days had no significant impact on bee mortality compared to control.

No effects on the behaviour of the bees (or other sublethal effects) were observed in comparison with the control bees.

Control bees:

The mortality in the control was 4 % for the house bees and 44 % for the field bees in the oral toxicity tests after 10 days.
The increasing mortality of the field bee control was observed starting with 14 % (day 7) up to 16 % (day 8), 30 % (day 9) and 44 % (day 10).

The validity criterion - mortality in the control ≤ 10 % - was accomplished for the whole test duration of 10 days for the house bee test (4 %) and for field bees up to day 6 (8 %) .

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1. General information

1.1. Objective

The purpose of this study was to determine the acute toxicity of Substance C on the honeybee *Apis mellifera* in a 10-day prolonged laboratory test of oral exposure.

1.2. Justification for selection of the test system

Data on the toxicity to *Apis mellifera* were generated to comply with the EU Registration Directive 91/414/EEC (amended by the Commission Directive 96/12/EC).
The selected test design corresponds to the recommendation of the EPPO Standard Guideline PP/1/170 (2) (1999). "Side-effects on honeybees" and the OECD Guidelines 213 (draft 1998).

1.3. Project staff

Study Director: [redacted]

Personnel: [redacted]

1.4. Time schedule

Study initiation date:	12.05.00
Signature of the study plan by the sponsor:	15.05.00
Experimental start date:	17.05.00
Experimental termination date:	28.05.00
Study completion date:	04.07.00

1.5. Test guidelines

EPPO Standard PP1/170 (2) (1999)
OECD Guideline No. 213 (draft 1998)

1.6. Archiving

Study plan and final report as well as the raw data will be kept in the archive of BioChem agrar GmbH Cunnersdorf.

2. Performance of the test

2.1. Test substance

Name.:	Substance C
Received:	25.04.2000
Active ingredient/content:	C not (available)
Appearance:	white powder
Stability:	chemically stable under standard conditions
Water solubility:	soluble (> 100 mg/l)
Storage conditions:	room temperature (max. 30 °C), dark and dry
Safety precautions:	designation according to "EU Classification": no information consideration of the safety measures in general use with handling of plant protection products
Field of use:	unknown
Dose applied in the test:	oral: 10, 1, 0.1 ppb (equivalent to 10, 1, 0.1 ng/g sucrose solution)
Further details:	fax of: 12.04.00, 19.04.00 and 25.04.00

2.2. Reference substance (toxic standard)

was not required

2.3. Control

The control was treated with 50 % (w/v) sucrose solution.

2.4. Test system

Test organism: honeybee - *Apis mellifera carnica* L.
(field and house bees of a colony in good health condition)

Origin of the test organism: purchased from the bee-keeper [redacted]
on 03.05.00

All bees used in the test came from healthy, disease free and queen-right bee colonies. The bees were taken from a hive that had not received treatments with chemical substances for at least one month. The honeybees were reared in the hive until they were used for testing.
10 bees were transferred to each test cage without anaesthesia. Field bees were collected from an entrance located on the top of the bee hive using an automatic trapping device (carousel with 4 glass tubes divided into two sections: a) ∅ 2 cm and 25 cm long; b) ∅ 0.5 cm and 10 cm long). For collecting the bees the carousel with the glass tubes was placed on the top of the bee hive. The glass tubes were fixed in the carousel. Then the entrance on the top of the bee hive was opened. After a bee walked in the glass tube, the carousel was turned so that the next empty glass tube was located over the entrance. The tube containing the bee was removed and placed on the test cage entrance. The bee was put inside the test cage by gentle blowing in the thinner end of the glass tube. This process was repeated until sufficient bees were available within the test cages for use in the test.
House bees were collected directly from a comb using a glass tubes divided into two sections: a) ∅ 2 cm and 25 cm long; b) ∅ 0.5 cm and 10 cm long). The bees walking in the glass tube glass the glass tube was closed. The tube containing the bee was than placed on the test cage entrance. The bee was put inside the test cage by gentle blowing in the thinner end of the glass tube. This process was repeated until sufficient bees were available within the test cages for use in the test.
After transferring the bees in the test cages they had time for acclimatisation to the test room conditions for ca. 2 hours (starving period before application of the bees).

Test conditions

Test cage: disposable cage 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)

Number of honeybees/cage: 10

Number of cages/concentration: 5

Number of honeybees/concentration: 50

Feeding: continuously during the test

Food: 50 % (w/v) aqueous sucrose solution

Climatic conditions (test room)

Temperature:	24-26 °C (according to EPPO Guideline and study plan: (25±2) °C)
Relative humidity:	63-75 % (according to EPPO Guideline and study plan: about 60-70 %)
Both registered:	continuously by a thermohygrograph Manufacturer: Feingerätebau Drebach type: 456
Illumination:	constant darkness throughout the test (with the exception of diffuse artificial light for less than 8 hours (over the whole test period) for the treatment and observations (ca 100 lx))

Test duration: 10 day

2.5. Experimental procedure

2.5.1. Preparation of the test solutions and method of application

Table 1: Test concentrations used in the test

treatment group	product	concentration ppb (ng/g sucrose solution)
1 control (oral)	50 % sucrose solution	-
2 test substance	Substance C	10 (10) 1 (1) 0.1 (0.1)

The 50 % (w/v) sucrose solutions was prepared with deionised water, just prior to application.

The exactly weighed out amounts of the substance were dispersed in sucrose solution (see appendix 1).

2.5.2. Course of the trial

Field and house-bees *Apis mellifera* L. were exposed to different doses of the test substance. The treated bees were kept under controlled climatic conditions and assessed for toxic effects for up to 10 days. For both tests control treatments were included in the test design. At the beginning of the test, 10 healthy workerbees per replicate (5 replicates/test concentration) were transferred individually in glass tubes from the hive (i.e. from the trapping device) to the test cage without anaesthesia. For both oral toxicity tests the groups of 10 healthy bees for use in different treatment groups were chosen without bias.
The bees were starved for a period of ca. 2 hours prior to test initiation in order that the bees were equal in terms of their gut contents at the start of the test. The preparation of the test solution was performed according to point 2.5.1. The feeding tube were filled up on day of first application with et least 2 ml of 50 % (w/v) aqueous sucrose solution containing the different test substance concentration. On the following days the feeding tubes were filled up again with 1-0.5 ml new sucrose solution containing the different test substance concentrations solution before the bees had consumed the whole sucrose solution. Food was provided *ad libitum* using a feeding tube (described in the section below). The amount of consumed sucrose solution on day 10 was determined for the initial number of bees on day of test initiation (10 bees/cage).
The number of dead and affected bees were counted at 4, 24, 48 hours and daily in 24 h intervals up to 10 days. At these times any behavioural abnormalities of the bees were also recorded.

Oral toxicity test

The bees were fed with a defined quantity of a 50 % aqueous sucrose solution that contained the test substance (in a concentration series). The control treatments were fed with 50 % aqueous sucrose solution.
The amount of consumed sucrose solution was determined by weighing the empty feeding tube (pre-weighed) and the filled feeding tubes (rest amount) on day 10.
Also the amount of food provided during the whole test duration was summed up (see appendix).
In the test, groups of 10 bees were provided with the test solution which was presented to the bees in a glass ampoule. The feeding tubes were introduced through a hole in the roof of the cage.
Due to their social feeding habit, the honeybees of a distinct group are assumed to receive approximately the same amount of the test substance. On day 10 after test beginning, the feeding tube was reweighed to determine the exact quantity of the test solution consumed.

Order of application and cleaning procedure

The order of feeding or topical application was the following:

- control
- test substance (from the lowest to the highest concentration)

Following application of the treatments, the equipment used was cleaned according to the respective SOP. Cleaning agents were "Essigreiniger", washing-up liquid, tap water and deionised water for glass equipment.

Chronological test schedule (abstract)

- Transfer of the bee hive to the test room
- Preparation of the test cages
- Transfer of 10 bees to each cage (without anaesthesia) and acclimatisation for approximately 2 hours in both tests
- In the acclimatisation phase of the oral toxicity test no food was provided before application (i.e. the bees were starved for ca. 2 hours)
- Preparation of the test solutions (up to 1 h before application)
- Application of the test solutions (oral administration)
- Filling up the food tubes with 50 % new sucrose solution containing the required test substance concentrations each day
- Observation of the bees throughout the experiment (every 24 hours) and feeding as required
- Final assessment 10 days after application
- On day 10 of the oral application test, feeding tubes were reweighed

2.5.3. Assessment of the effects

Time and frequency of assessments:
· 4, 24, 48 hours and daily up to day 10 after application

Evaluation parameters:
· mortality: number of dead bees per cage
· behaviour: poisoning symptoms and behavioural anomalies in comparison with the control bees

Validity criterion:
· mortality in the control: ≤ 10 %

2.6. Calculation and evaluation

The corrected mortality according to ABBOTT was calculated for each concentration following the formula

$$M\,(\%) = \frac{c - t}{c} \cdot 100$$

M	=	corrected mortality (%)
c	=	number of surviving bees in the control treatment group
t	=	number of surviving bees in the treated treatment group

The determination of the statistical significance was carried out by FISCHER exact-test (EASY ASSAY Critical Values Ver. 3.01, 1998 by RATTE).

3. Results

The findings are summarized in table 2 and the detailed set of results are presented in Appendices 3 and 4.

4 % mortality was observed for house bees in the control treatments on day 10. Thus, the test accomplished the validity criterion (mortality in the control ≤ 10 %).
44 % mortality was observed for field bees in the control treatments on day 10. Thus, the test accomplished not the validity criterion (mortality in the control ≤ 10 %) until test day 10. The test accomplished the validity criterion (mortality in the control ≤ 10 %) only until test day 6 with 8 % mortality.
Oral exposure of substance C to field and house bees resulted in no significant mortality at concentrations of 0.1, 1.0 up to 10.0 ppb test substance containing sucrose solution per bee provided over the whole test duration of 10 days.

The tested concentrations of 0.1, 1.0 and 10 ppb test substance C containing sucrose solution resulted in 10, 4 and 6 % mortality after oral exposure to house bees over the whole test period of 10 days.
The field bees showed also no statistically significant differences in mortality, after oral exposure of tests substance C at concentrations of 0.1, 1.0 and 10 ppb, compared to control
The oral exposure of test substance concentration of 0.1, 1.0 and 10 ppb resulted in 30, 40, and 32 % mortality.
The consumed amount of contaminated sucrose solutions for each dose level was determined on day 10. The results in terms of the actual measured consumed dose are presented in appendix 6.

No effects on behaviour of bees were observed for any test substance dose level after oral exposure of test substance C.

Table 2: Mortality of bees in the oral toxicity test with Substance C (group feeding)

treatment	Concentration/Dose (ppb/ng/bee*)	mortality (%)	corrected mortality (%) (according to ABBOTT)
		on day 10 of continuous application	
Control (sucrose solution)		4	-
Substance C house bees	10 (7.242) 1 (0.734) 0.1(0.072)	6 4 10	2.1 0 6.3
Control (sucrose solution)		44	-
Substance C field bees	10 (8.056) 1 (0.818) 0.1(0.083)	32 40 30	-21.7 -7.1 –25.0

* calculated from the quantity of test solution remaining after 10 days (see appendix 6)

4. Interpretation

Oral exposure of house and field bees to test substance C resulted in no significant effects on honeybee mortality at test concentrations of 0.1, 1 and 10 ppb per bee provided over 10 days diluted in sucrose solution.

The validity criterion - mortality in the control ≤ 10 % - was accomplished for whole test duration of 10 days for the house bee test (4 %) and for field bees up to day 6 (8%) .

5. References

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OECD Guidelines For The Testing of Chemicals (1998):
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EASY ASSAY, *Critical Values*, Ver. 3.01, 1998.
SpiRiT, Aachen 1992-1998.

6. Signatures

Confirmation of the final report

Study Director: [redacted] 24.07.05
Date

Testing Facility Management: [redacted] 04.07.05
Date

Sponsor/Monitor: [redacted] 19.7.05
Date

Appendix 1: Preparation of test solutions - oral toxicity test

1. *Preparation of sucrose solution (= control)*
- Dissolving of the appropriate amount of sucrose in deionised water which gave a 50 % w/v sucrose solution; density of the 50 % aqueous sucrose solution: 1.220 g/cm^3

2. *Preparation of test substance solutions*
- Weighed: 50 mg substance C
- Dissolving in 500 ml deionised water which gave the stock solution A (100 ppm)
- 1 ml stock solution A made up to 100 ml which gave stock solution B (1 ppm)
- Preparation of dilutions:

Volume of solution	name	+ 50 % sucrose solution	total volume of solution		Concentration (ppb)	concentration (ng/g sucrose solution)
1 ml (1 g)	B	Made up to	= 100 g	C	10	10
10 g	C	Made up to	= 100 g	D	1	1
10 g	D	Made up to	= 100 g	E	0.1	0.1

The solutions C to E were used for dosing.

Appendix 2: Table of results – Surviving house bees

house bees					
Substance C					
study day	replicate	number of surviving bees			
		control	10 ppb	1 ppb	0.1 ppb
1	1	10	10	10	9
	2	10	10	10	10
	3	10	10	10	10
	4	10	10	10	10
	5	10	10	10	10
2	1	10	10	10	9
	2	10	10	10	10
	3	10	10	10	10
	4	10	10	10	10
	5	10	10	10	10
3	1	10	10	10	9
	2	10	10	10	10
	3	10	10	10	10
	4	10	10	10	10
	5	10	10	10	10
4	1	10	10	10	9
	2	10	10	10	10
	3	10	10	10	10
	4	10	10	10	10
	5	10	10	10	10
5	1	10	10	10	9
	2	10	10	10	10
	3	10	10	10	10
	4	10	10	10	10
	5	10	10	10	10
6	1	10	10	10	9
	2	10	10	10	10
	3	10	10	10	10
	4	10	10	10	9
	5	10	10	9	10
7	1	10	10	10	9
	2	10	10	10	10
	3	10	10	10	9
	4	10	10	10	9
	5	10	10	9	10
8	1	10	9	10	9
	2	10	10	10	10
	3	10	10	10	9
	4	10	10	10	9
	5	10	10	9	10
9	1	10	9	10	9
	2	10	10	10	10
	3	10	10	10	8
	4	9	10	10	9
	5	10	9	9	10
10	1	10	9	9	9
	2	9	9	10	10
	3	10	10	10	8
	4	9	10	10	9
	5	10	9	9	9

Appendix 3: Table of results: Mortality - house bees

treatment	replicate	concentration of test solution (ppb)	consumed test solution per 10 bees (g)	substance per bee (ng)	surviving bees per cage (day 10)	Mortality %	corrected according to ABBOTT (%)
control (sucrose solution)	1		6.686		10		
	2		7.592		9		
	3		7.035		10		
	4		7.341		9		
	5		7.825		10		
			7.2958				
	mean				48	4	-
Substance C	1	10	7.343	7.343	9		
	2		6.554	6.554	9		
	3		7.696	7.696	10		
	4		7.221	7.221	10		
	5		7.395	7.395	9		
	mean		7.242	7.242	47	6	2.1
	1	1	7.510	0.751	9		
	2		7.340	0.734	10		
	3		7.823	0.782	10		
	4		6.792	0.679	10		
	5		7.243	0.724	9		
	Mean		7.342	0.734	48	4	0
	1	0.1	7.264	0.073	9		
	2		6.432	0.064	10		
	3		6.755	0.068	8		
	4		7.505	0.075	9		
	5		7.997	0.080	9		
	mean		7.191	0.072	45	10	6.3

Appendix 4: Table of results – surviving field bees

field bees					
Substance C					
study day	replicate	number of surviving bees			
		control	10 ppb	1 ppb	0.1 ppb
1	1	10	10	10	10
	2	10	10	10	10
	3	10	10	9	10
	4	10	10	10	10
	5	10	10	10	10
2	1	10	10	10	10
	2	10	10	10	10
	3	10	10	9	10
	4	10	10	10	10
	5	10	10	10	10
3	1	10	10	10	10
	2	10	10	10	10
	3	10	9	9	10
	4	10	10	10	10
	5	10	9	9	10
4	1	10	10	10	10
	2	10	10	10	10
	3	10	9	9	10
	4	10	9	10	10
	5	10	9	8	10
5	1	10	10	10	10
	2	10	8	10	10
	3	10	9	9	10
	4	10	9	10	9
	5	10	8	8	10
6	1	7	10	10	10
	2	10	7	10	10
	3	10	9	8	10
	4	10	9	10	9
	5	9	7	7	10
7	1	7	10	9	10
	2	7	7	10	10
	3	10	8	6	9
	4	10	9	10	8
	5	9	6	7	10
8	1	7	10	8	7
	2	6	7	10	10
	3	10	8	5	9
	4	10	9	9	7
	5	9	4	7	9
9	1	7	7	5	6
	2	6	6	8	10
	3	7	8	5	5
	4	9	9	6	7
	5	6	4	6	9
10	1	5	7	5	6
	2	5	6	8	9
	3	5	8	5	5
	4	8	9	6	7
	5	5	4	6	8

Appendix 5: Table of results: Mortality - field bees

treatment	replicate	concentration of test solution (ppb)	consumed test solution per 10 bees (g)	substance per bee (ng)	surviving bees per cage (day 10)	mortality %	corrected according to ABBOTT (%)
control (sucrose solution)	1		8.385		5		
	2		6.908		5		
	3		8.793		5		
	4		8.000		8		
	5		7.631		5		
	mean		7.943		28	44	-
Substance C	1	10	8.025	8.025	7		
	2		7.523	7.523	6		
	3		8.41	8.410	8		
	4		9.147	9.147	9		
	5		7.176	7.176	4		
	mean		8.056	8.056	34	32	- 21.4
	1	1	8.972	0.897	5		
	2		9.224	0.922	8		
	3		7.257	0.726	5		
	4		8.143	0.814	6		
	5		7.282	0.728	6		
	Mean		8.176	0.818	30	40	- 7.1
	1	0.1	8.825	0.088	6		
	2		8.887	0.089	9		
	3		8.356	0.084	5		
	4		7.225	0.072	7		
	5		8.169	0.082	8		
	mean		8.292	0.083	35	30	- 25.0

Appendix 6: Consumed sucrose solution (g/day)/ consumed test substance (ng/bee)

House bee

treatment	replicate	Amount sucrose solution provided 10 bees (ml)									Σ	Σ	rest/ tube	Σ	ng C/ bee
	day	1	2	3	4	5	6	7	8	9	ml	g	g	g	
control	W1	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.854	6.686	0.669
	W2	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	0.948	7.592	0.759
	W3	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.505	7.035	0.704
	W4	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.199	7.341	0.734
	W5	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	0.715	7.825	0.783
	mean													**7.296**	0.730
10 ppb	W1	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.197	7.343	7.343
	W2	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.986	6.554	6.554
	W3	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	0.844	7.696	7.696
	W4	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.319	7.221	7.221
	W5	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.145	7.395	7.395
	mean													7.242	7.2418
1.0 ppb	W1	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.03	7.51	0.751
	W2	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.2	7.34	0.734
	W3	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	0.717	7.823	0.782
	W4	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.748	6.792	0.679
	W5	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.297	7.243	0.724
	mean													**7.342**	**0.7342**
0.1 ppb	W1	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.276	7.264	0.073
	W2	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	2.108	6.432	0.064
	W3	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.785	6.755	0.068
	W4	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	1.035	7.505	0.075
	W5	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7	8.54	0.543	7.997	0.080
	mean													**7.191**	**0.0719**

Field bee

treatment	replicate	Amount sucrose solution provided 10 bees on study day (ml)										Σ	Σ	rest/ tube	Σ	ng C/ bee
	day	1		2	3	4	5	6	7	8	9	ml	g	g	g	
control	W1	0.919	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.919	9.661	1.276	8.385	0.839
	W2	0.775	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.775	9.485	2.577	6.908	0.691
	W3	0.962	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.962	9.714	0.921	8.793	0.879
	W4	0.762	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.762	9.47	1.47	8	0.800
	W5	0.841	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.841	9.566	1.935	7.631	0.763
	mean														**7.94**	**0.794**
10 ppb	W1	0.847	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.847	9.573	1.548	8.025	8.025
	W2	0.772	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.772	9.482	1.959	7.523	7.523
	W3	0.788	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.788	9.501	1.091	8.41	8.410
	W4	0.767	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.767	9.476	0.329	9.147	9.147
	W5	0.819	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.819	9.539	2.363	7.176	7.176
	mean														**8.06**	**8.056**
1.0 ppb	W1	0.832	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.832	9.555	0.583	8.972	0.897
	W2	0.744	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.744	9.448	0.224	9.224	0.922
	W3	0.769	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.769	9.478	2.221	7.257	0.726
	W4	0.864	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.864	9.594	1.451	8.143	0.814
	W5	0.741	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.741	9.444	2.162	7.282	0.728
	mean	0.790													**8.18**	**0.818**
0.1 ppb	W1	0.823	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.823	9.544	0.719	8.825	0.088
	W2	0.821	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.821	9.542	0.655	8.887	0.089
	W3	0.750	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.750	9.455	1.099	8.356	0.084
	W4	0.751	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.751	9.456	2.231	7.225	0.072
	W5	0.791	2	1	1	0.5	0.5	0.5	0.5	0.5	0.5	7.791	9.505	1.336	8.169	0.082
	mean	0.787													**8.29**	**0.083**

Amendment to Report No. 00 10 48 502c

Identification of test substance

Code name in report:	Test substance C
Name of test substance:	6-Chloronicotinic acid
Origin of test substance:	Bayer AG, Leverkusen PF-F/FT-EA
Specification	
Substance no.	870922ELB06
a.i. content:	99,6 %
Date of analysis:	8.8.1995
Expiry date:	1.8.2000
Delivered to:	Bayer AG Institute for Environmental Biology Laboratory for non-target arthropods Internal laboratory no. 220
Date of reception:	13.4.2000
Contract laboratory:	Biochem GmbH, Cunnersdorf
Date of delivery as substance C:	18.4.2000
Delivered amount:	0.23 g
Order no.:	347517E0

Leverkusen, 21.6.00

[redacted]