



Substance C

Final Report

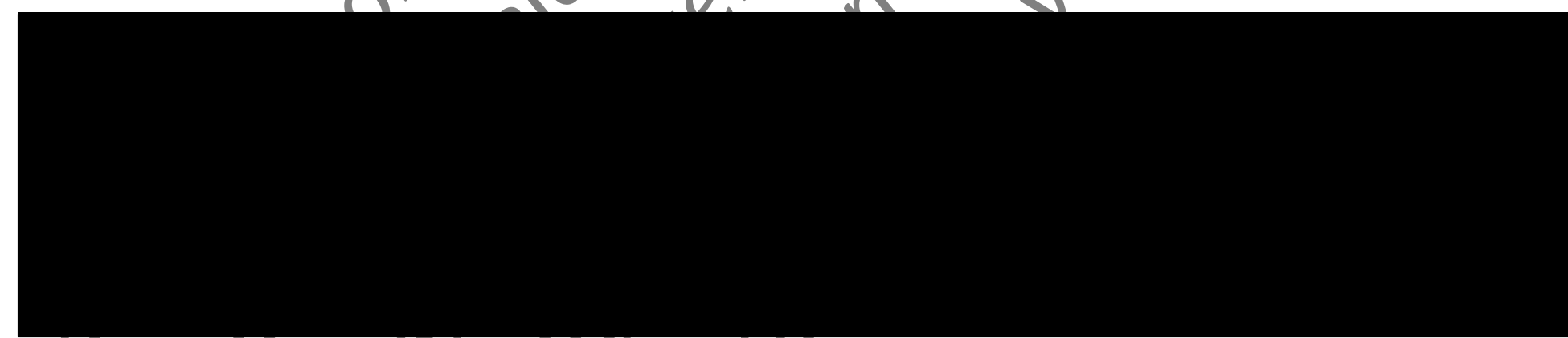
20001149/01-BLEU

Final Report

**Substance C:
Assessment of Side Effects in a Ten Days Feeding Test
on the Honey Bee, *Apis mellifera* L.
in the Laboratory**

**foraging bees
(= 22-32 days)**

Study Director



Date

13/06/2000

Testing facility

Arbeitsgemeinschaft
GAB Biotechnologie GmbH &
IFU Umweltanalytik GmbH
Eutingen Str. 24
D-75223 Niefern-Öschelbronn
Germany

Sponsor

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Geschäftsbereich Pflanzenschutz
Alfred-Nobel-Str. 50
40789 Monheim
Germany



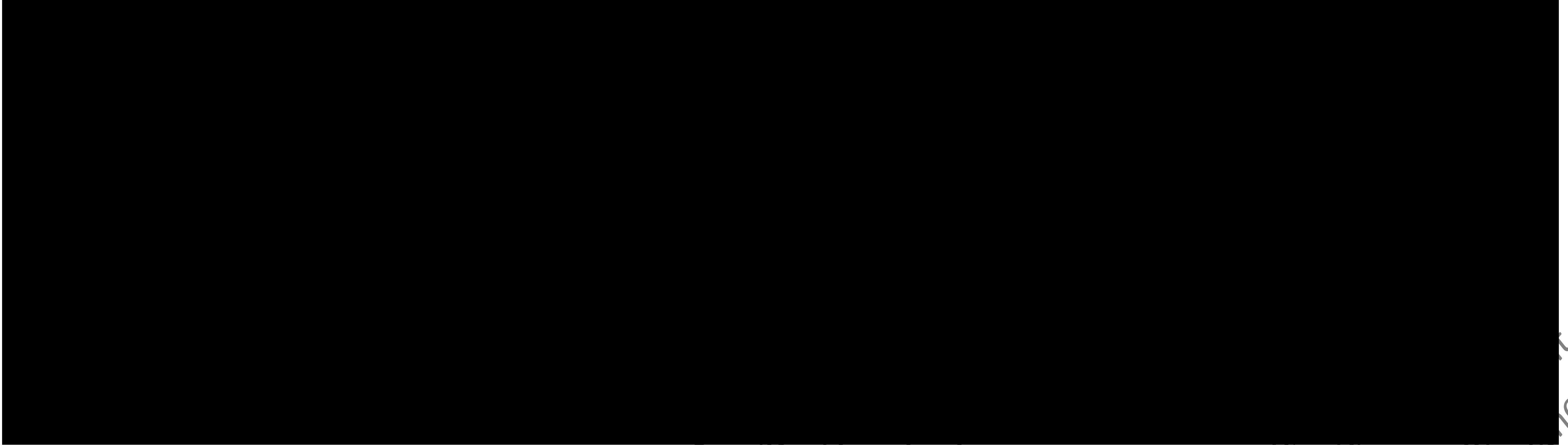
20001149/01-BLEU 2. / MO-02-008343

Study Identification Code

Test substance: Substance C
Study code: 20001149/01-BLEU



Approval Page



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1 Summary

Worker honey bees (age: approx. 22 - 32 days) were fed over a four days period with sucrose solution mixed with Substance C. The feeding test was carried out with three different concentrations of the test substance and with five replicates. Due to a high mortality which occurred in the control group the test was terminated after four days instead of a ten days exposure period.

In the treatment with Substance C the mortality rose up to 10 % observed at a test substance concentration of 1 µg/L after four days.

A 6 % mortality occurred in the treatment group fed with the highest concentrated test substance solution (10 µg/L) of Substance C (actual intake 2.731 ng/bee).

In the control group a 20 % mortality was observed after the four days exposure period.

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2 Material and Methods

2.1 Test item and control

Test Item

Name: Substance C
 GAB-Code: 20001149
 Appearance / Colour: powder / white
 Density: not relevant
 Solubility: in water
 Stability: test item must be considered as stable under test conditions
 Storage of the test solutions: 4°C, dark

Control

50 % (w/v) sucrose solution

2.2 Test organism

Taxonomic Group: honey bees (Insecta, Hymenoptera)
 Species: adult *Apis mellifera carnica* L.
 Age: age: approx. 22 - 32 days

2.3 Test units

Type: cages made of high grade steel
 Size: width: 10 cm; depth: 5.5 cm; height: 8.5 cm
 Front side: transparent glass-pane
 Bottom: perforated board
 Inner walls: lined with filter paper

2.4 Test conditions

Temperature:	24 - 28°C
Humidity:	45 – 68 %
Light:	darkness

2.5 Application of the test item and the control

Dosage of the test item	0.1 µl and 10 µg/L of Substance C food (50 % sucrose solution) was mixed with a definite amount of the test substance and offered in syringes (Braun inject; 5 ml) which were weighed before and after introduction into the cages.
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2.6 Course of the test

Treatment groups:	control (age: approx. 22 - 32 days) 3 doses of the test item tested with bees (age: approx. 22 - 32 days)
Replicates:	5 per treatment group
Exposure period:	10 days

2.7 Food

syringes with food were changed on day + 3

2.8 Test Parameters

Mortality	number of dead bees were recorded every day
Food uptake	food uptake was be recorded every day by weighing the syringes
Behavioural Abnormalities:	behavioural abnormalities were recorded at every assessment date

2.9 Results

The average mortality in all treatment groups and in the control and the respective actual intake of the test substance Substance C after a four days exposure are presented in Table 1.

Table 1: Average mortality on exposure day +4 in the feeding test with Substance C as a function of the intake of test substance and the control

Treatment	Concentration [µg/L]	Intake of test substance solution [g/bee]	Intake of test substance [ng/bee]	Mortality [%]
Control	-	0.458	-	20
Substance C	0.1	0.352	0.0301	8
	1	0.389	0.3322	10
	10	0.320	2.7310	6

Weight of sucrose solution: 2.17 mg/ml

In the treatment with Substance C the mortality rose up to 10 % observed at a test substance concentration of 1 µg/L after four days.

A 6 % mortality occurred in the treatment group fed with the highest concentrated test substance solution (10 µg/L) of Substance C (actual intake: 2.731 ng/bee).

In the control group a 20 % mortality was observed after the four days exposure period.

Due to a high mortality which occurred in the control group the test was terminated after four days exposure period.

3 Appendix

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GAB Calculation Sheet for Honey Bee Laboratory Tests (EPPO)

Calculation of the intake of test substance

Study code: Bayer non GLP-Bienerversuch

Test substance: Kontrolle

Date: 10/05/2000

Density of sucrose solution: 1.7

Date	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average intake [mg]	Weight after feeding [g]	Intake [g]	Average intake [mg]	Weight after feeding [g]	Intake [g]	Average intake [mg]
10/05/2000	7.536	6.076	1.460		5.029	1.047		4.220	0.809	
Control	7.556	6.363	1.193		5.261	1.102		4.318	0.943	
	7.423	6.173	1.250		5.394	0.678		4.965	0.529	
	7.509	6.331	1.178		5.666	0.665		5.086	0.580	
	7.668	6.243	1.425	1.301	5.147	1.098	1.098	4.098	1.049	0.782

Date	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average intake [mg]
13/05/2000	7.475	6.628	1.040	
Control	7.485	6.601	1.167	
	7.533	6.954	0.714	
	7.466	6.763	0.906	
	7.480	6.475	1.183	1.004

Amendment to Report No. 20001149/01-BLEU

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

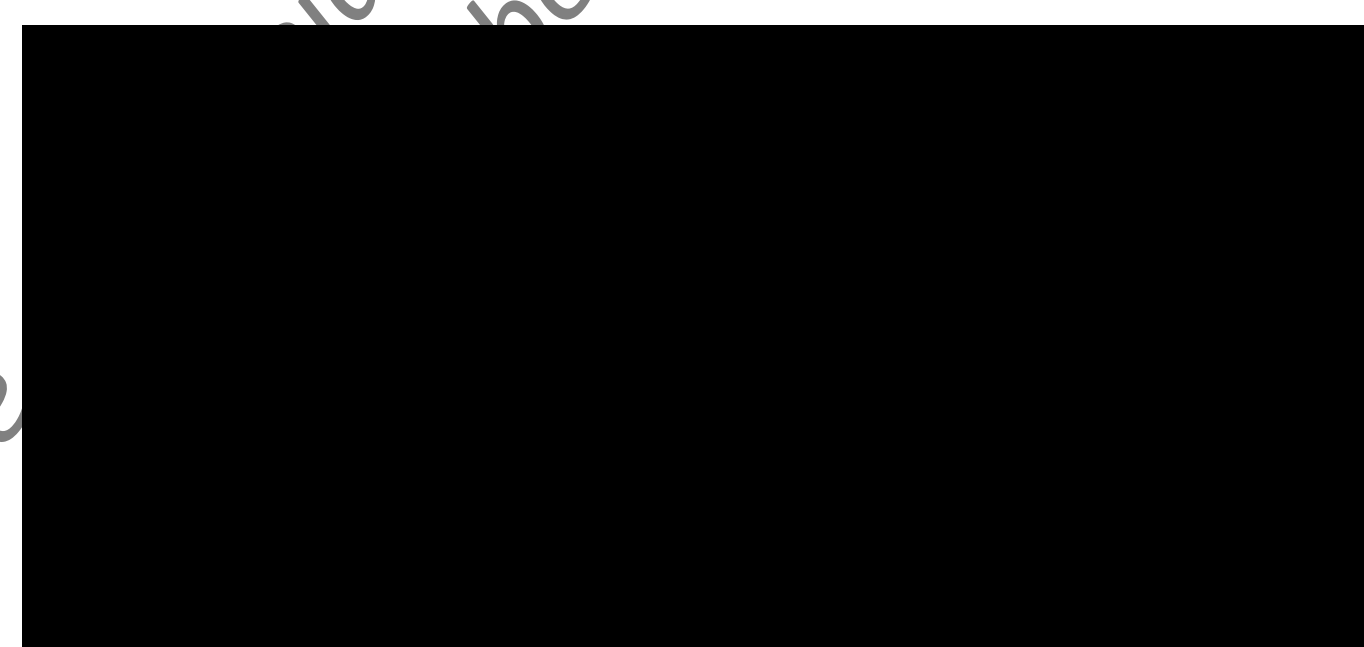
Delivered to: Bayer AG
Institute for Environmental Biology
Laboratory for non-target arthropods
Internal laboratory no. 220

Date of reception: 13.4.2000

Contract laboratory: GAB/ Biotechnologie, Niefern-Öschelbrunn

Date of delivery as substance C: 14.4.2000
Delivered amount: 0.23 g
Order no.: 337669 K

Leverkusen, 21.6.00



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