



Substance C

Final Report

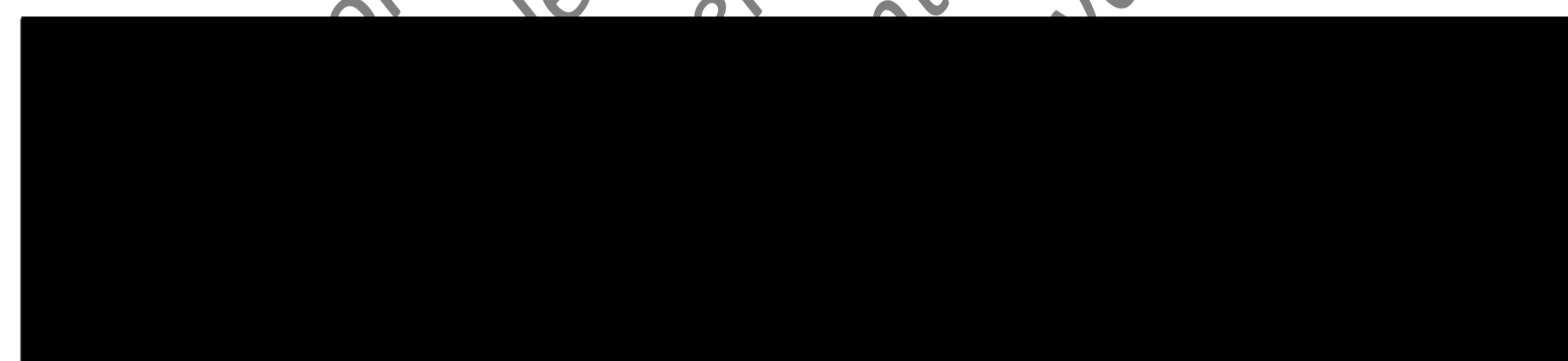
20001149/01-BLEU

## Final Report

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

## hive bees ( $\leq 5$ days)

Study Director



Date

09/06/2000



20001148/01-BLEU 3. / MO-02-008340

### Testing facility

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

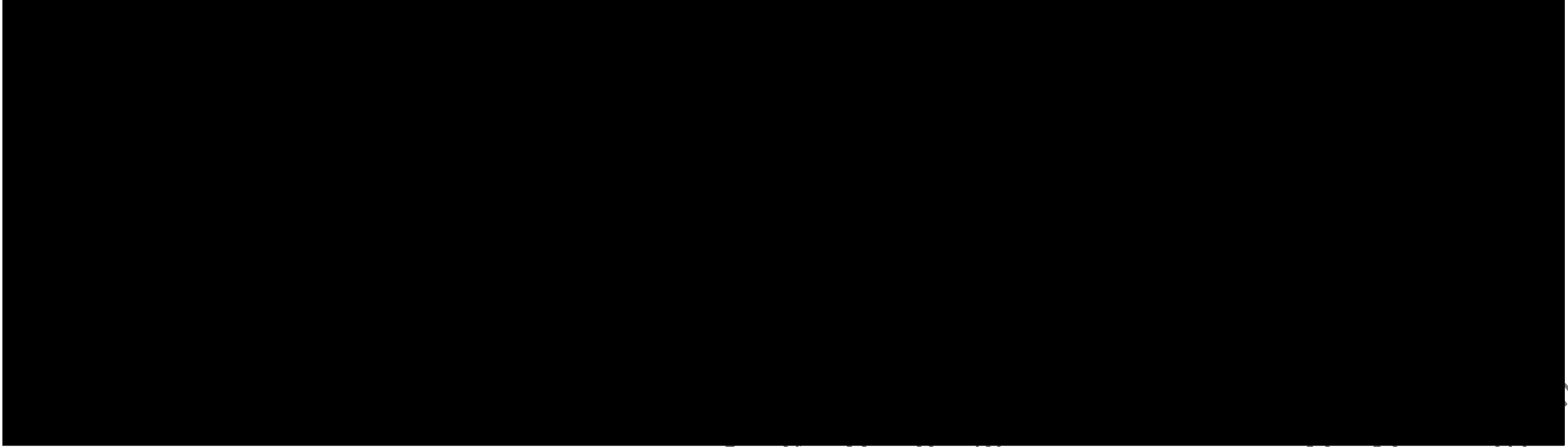
### Sponsor

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

### Study Identification Code

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

## Approval Page



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## 1 Summary

Young honey bees (1-5 days old) were fed over a ten 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.

To obtain bees of approx. the same age, combs with bee brood, deriving from a healthy colony, were incubated in the laboratory for five days. The bees which hatched within five days were used for this feeding test. The young bees only fed the honey which was found in the combs, until the test started.

In the treatments with Substance C the mortality rose up to 4 % observed at a test substance concentration of 1 µg/L (actual intake: 0.4585 ng/bee) after 10 days.

No mortality occurred in the treatment group fed with the highest concentrated test substance solution (10 µg/L) of Substance C (actual intake: 4.6769 ng/bee).

No mortality was observed in the control group after the ten 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: up to 5 days old. To obtain bees of approx. the same age, combs with bee brood, deriving from a healthy colony, were incubated in the laboratory for five days. The bees which hatched within five days were used for this feeding test. The young bees only fed the honey which was found in the combs, until the test started.

### 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 1 – 5 days old) 3 doses of the test item tested with bees (age 1 – 5 days old)
Replicates:	5 per treatment group
Exposure period:	10 days

## 2.7 Food

syringes with food were changed on day + 3; + 6 and +8 of the ten days exposure period

## 2.8 Test Parameters

Mortality	number of dead bees were recorded every day in the first four days and every second day in the following days. On every assessment date the dead bees were removed from the test cages
Food uptake	food uptake was be recorded every day in the first four days and every second day in the following days 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 ten days exposure are presented in Table 1.

Table 1: Average mortality on exposure day +10 in the ten days 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	-	0.5512	-	0
Substance C	0.1	0.5265	0.04500	2
	1	0.5895	0.45850	4
	10	0.5472	4.67692	0

Weight of sucrose solution: 2.17 mg/ml

In the treatment with Substance C the mortality rose up to 4 % observed at a test substance concentration of 1 µg/L after 10 days.

No mortality occurred in the treatment group fed with the highest concentrated test substance solution (10 µg/L) with Substance C (actual intake: 4.6769 ng/bee).

No mortality was observed in the control group after the ten 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-Bienenversuch  
 Test substance: Kontrolle  
 Date: 10/05/2000  
 Density of sucrose solution: 1,17

Date	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Weight after feeding [g]	12/05/2000	13/05/2000	Intake [g]	Average Intake [g]
Control	7,520	7,098	0,422	0,505	6,593	6,038	0,555	0,555	0,459
	7,513	7,119	0,394	0,276	6,843	6,531	0,312	0,312	
	7,488	6,911	0,578	0,579	6,332	5,806	0,526	0,526	
	7,508	6,987	0,521	0,496	6,491	5,914	0,577	0,577	
	7,667	7,258	0,429	0,489	6,769	6,443	0,326	0,326	0,459

Date	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Weight after feeding [g]	16/05/2000	Intake [g]	Average Intake [g]
Control	7,484	6,952	0,532	0,735	5,426	1,526	1,526	1,364
	7,524	6,983	0,541	0,704	5,988	1,295	1,295	
	7,517	7,031	0,486	0,656	5,478	1,553	1,553	
	7,491	7,132	0,361	0,555	6,002	1,130	1,130	
	7,480	7,029	0,451	0,658	5,112	1,317	1,317	1,364

Date	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]
Control	7,683	6,660	1,027	0,937
	7,668	6,939	0,748	
	7,694	6,534	1,153	
	7,674	6,866	0,821	
	7,692	6,588	1,099	0,937

Date	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]
Control	7,535	5,847	1,840	1,358
	7,568	6,974	0,713	
	7,628	5,783	1,904	
	7,715	6,315	1,372	
	7,600	6,725	0,962	1,358



# Amendment to Report No. 20001149/01-BLEU

## Identification of test substance

Code name in report:  
Name of test substance:

Test substance C  
6-Chloronicotinic acid

Origin of test substance:

Bayer AG, Leverkusen  
PF-F/FT-EA

### Specification

Substance no.  
a.i. content:  
Date of analysis:  
Expiry date:

870922ELB06  
99,6 %  
8.8.1995  
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

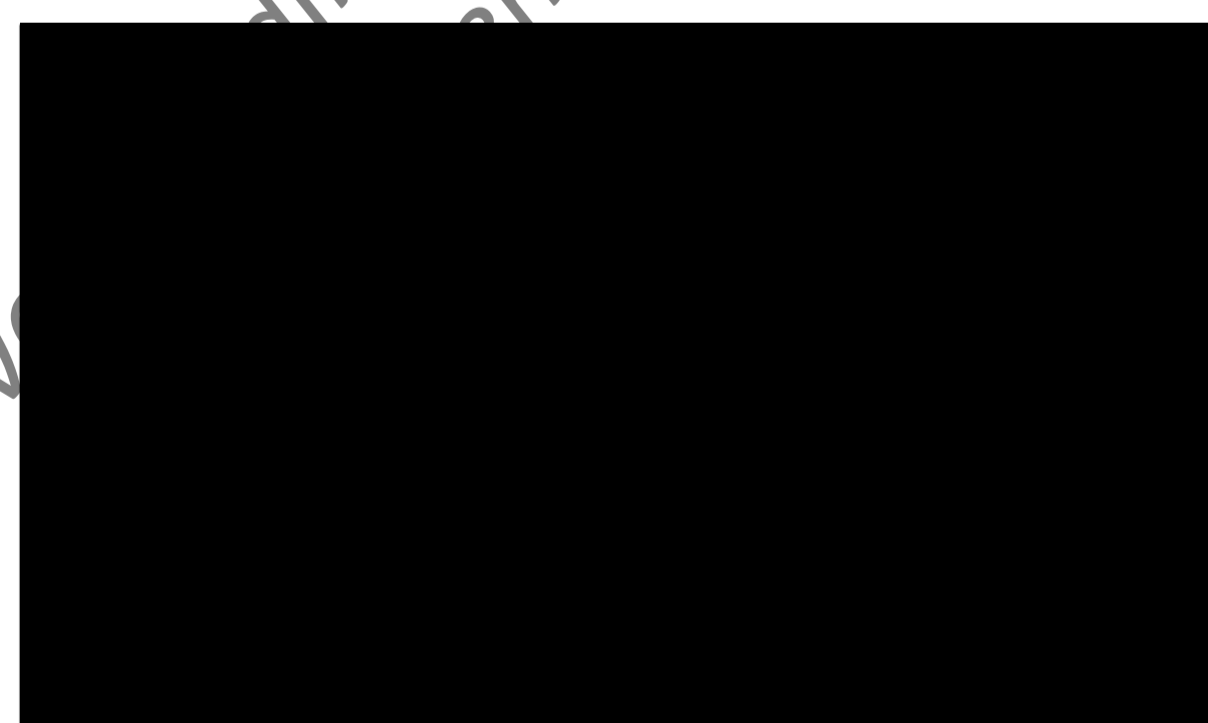
Delivered amount:

0.23 g

Order no.:

337669 K

Leverkusen, 21.6.00



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