



Substance B

Final Report

20001148/01-BLEU

## Final Report

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

Study Director



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

Date

13/06/2000

### Testing facility

Arbeitsgemeinschaft

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### Sponsor

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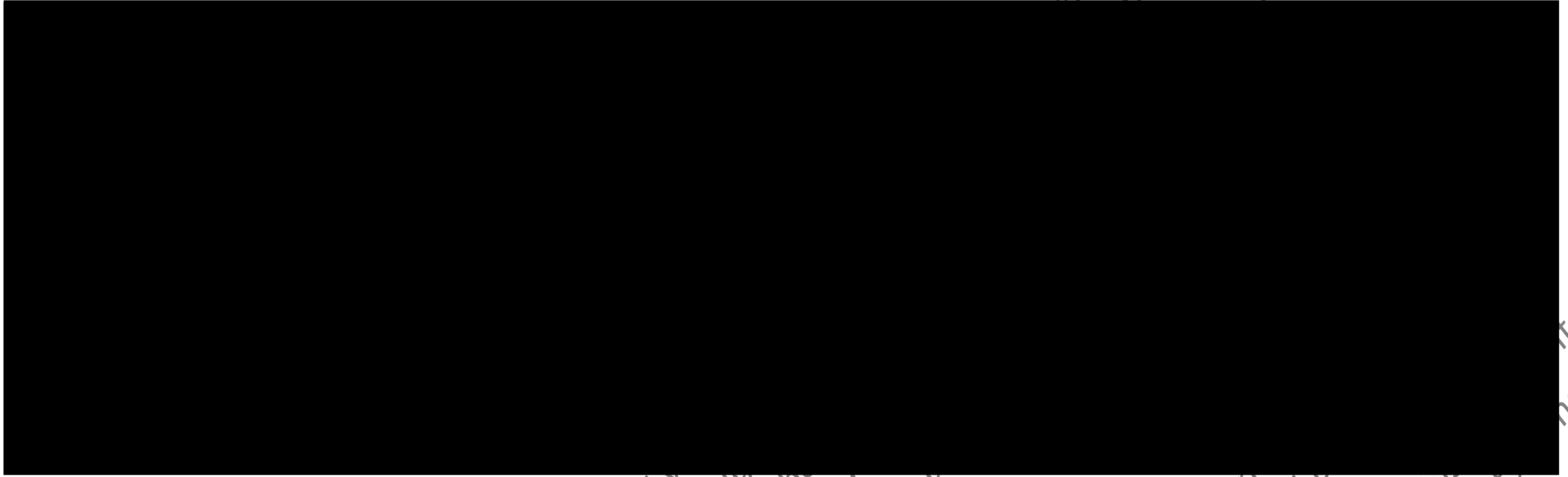
20001148/01-BLEU / MO-02-008310

### Study Identification Code

Test substance: Substance B

Study code: 20001148/01-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 B. 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.

The mortality in the Substance B treatment group rose up to 34 %, observed in the treatment fed with the lowest concentrated test substance solution of 0.1 µg/L which corresponded to an actual intake of 0.02873 ng/bee after four days.

A 16 % mortality occurred in the treatment group fed with the highest concentrated test substance solution (10 µg/L) of Substance B (actual intake: 2.881 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 B
GAB-Code	20001148
Appearance / Color:	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 worker <i>Apis mellifera carnica</i> 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, 1 and 10 µg/L of Substance B 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. On every assessment date the dead bees were removed from the test cages
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 B after a four days exposure are presented in Table 1.

Table 1: Average mortality on exposure day +4 in the feeding test with Substance B 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 B	0.1	0.336	0.02873	34
	1	0.316	0.27041	20
	10	0.337	2.88061	16

\*Weight of sucrose solution: 1.17 mg/ml

The mortality in the treatment groups with Substance B rose up to 34 %, observed in the treatment fed with the lowest concentrated test substance solution of 0.1 µg/L which corresponded to an actual intake of 0.0287 ng/bee after ten days.

A 16 % mortality occurred in the treatment group fed with the highest concentrated test substance solution (10 µg/L) of Substance B (actual intake: 2.88061 ng/bee).

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

### 3 Appendix

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**GAB Calculation Sheet for Honey Bee Laboratory Tests**

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

	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]
Date	10/05/2000	11/05/2000				12/05/2000			13/05/2000	
Control	7,536	6,076	1,460		5,029	1,047		4,220	0,809	
	7,556	6,363	1,193		5,261	1,102		4,318	0,943	
	7,423	6,173	1,250		5,494	0,679		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	1,096	0,918	4,098	1,049	0,782

	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [mg]
Date	13/05/2000	14/05/2000		
Control	7,475	6,628	1,040	
	7,485	6,501	1,167	
	7,533	6,954	0,714	
	7,466	6,763	0,905	
	7,480	6,475	1,193	1,004

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Substance B

Final Report

20001148/01-BLEU



**GAB Calculation Sheet for Honey Bee Laboratory Tests**

Calculation of the intake of test substance  
 Study code: 20001148/01-BLEU  
 Test substance: Substance E Fluginen  
 Date: 10/05/2000  
 Density of sucrose solution: 1,17

	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Average Intake [ $\mu\text{g}/\text{bee}$ ]	Sum Intake of test substance [ $\mu\text{g}/\text{bee}$ ]	Weight before feeding [g]	Intake [g]	Average Intake [g]	Average Intake [ $\mu\text{g}/\text{bee}$ ]	Sum Intake of test substance [ $\mu\text{g}/\text{bee}$ ]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Average Intake [ $\mu\text{g}/\text{bee}$ ]	Sum Intake of test substance [ $\mu\text{g}/\text{bee}$ ]
Date	10/05/2000	11/05/2000					12/05/2000					13/05/2000				
Concentration	7.535	6.563	0.972				5.828	1.186				5.172	0.756			
[g/L]	7.550	6.834	0.716				6.247	0.387				5.850	0.397			
[ $\mu\text{g}/\text{L}$ ]	7.735	6.723	1.012				6.105	0.618				5.828	0.276			
	7.618	6.723	0.895				6.159	0.584				5.475	0.684			
	7.750	6.746	1.004	0.960	0.0000683900	0.000023900	6.159	0.587	0.668	0.000066388	0.000150289	5.535	0.624	0.547	0.000067116	0.000211805
Concentration	7.585	6.634	0.951				6.083	0.551				5.601	0.582			
[g/L]	7.599	6.601	0.997				5.992	0.509				5.264	0.729			
	7.537	6.528	1.009				5.808	0.720				5.420	0.655			
	7.741	6.761	0.980				6.176	0.585				5.591	0.655			
	7.711	6.788	0.923	0.972	0.0000883797	0.0000883797	6.176	0.612	0.915	0.0000559193	0.0001442990	5.475	0.701	0.653	0.000067133	0.0002063123
Concentration	7.667	6.621	1.046				5.987	0.634				5.170	0.811			
[g/L]	7.582	6.416	1.166				5.852	0.564				5.504	0.518			
	7.556	6.581	0.975				5.790	0.791				5.632	0.767			
	7.595	6.682	0.913				6.044	0.638				5.445	0.599			
	7.588	6.347	1.241	1.066	0.0009821962	0.0009921962	5.620	0.727	0.871	0.000233742	0.0015165704	5.973	0.647	0.616	0.0006718346	0.0022874050

	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Average Intake [ $\mu\text{g}/\text{bee}$ ]	Sum Intake of test substance [ $\mu\text{g}/\text{bee}$ ]
Date	13/05/2000	14/05/2000				
Concentration	7.491	6.715	0.776			
[g/L]	7.439	7.049	0.390			
	7.457	7.154	0.303			
	7.424	6.828	0.796			
	7.453	6.805	0.648	0.583	0.0000075499	0.0000287303
Concentration	7.456	6.885	0.561			
[g/L]	7.503	6.757	0.746			
	7.479	6.928	0.551			
	7.412	6.809	0.603			
	7.474	6.934	0.540	0.600	0.0000641026	0.0002704118
Concentration	7.411	6.903	0.502			
[g/L]	7.376	6.777	0.599			
	7.439	6.654	0.765			
	7.479	7.019	0.459			
	7.469	6.886	0.583	0.583	0.0005932031	0.0028806081

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# Amendment to Report No. 20001148/01-BLEU

## Identification of test substance

Code name in report: Test substance B  
Name of test substance: Urea NTN33893

Origin of test substance: Bayer AG, Leverkusen  
PF-F/FT-EA

Specification  
Substance no. 960424ELB01  
a.i. content: 99,4 %  
Date of analysis: 13.4.2000  
Expiry date: April 2002

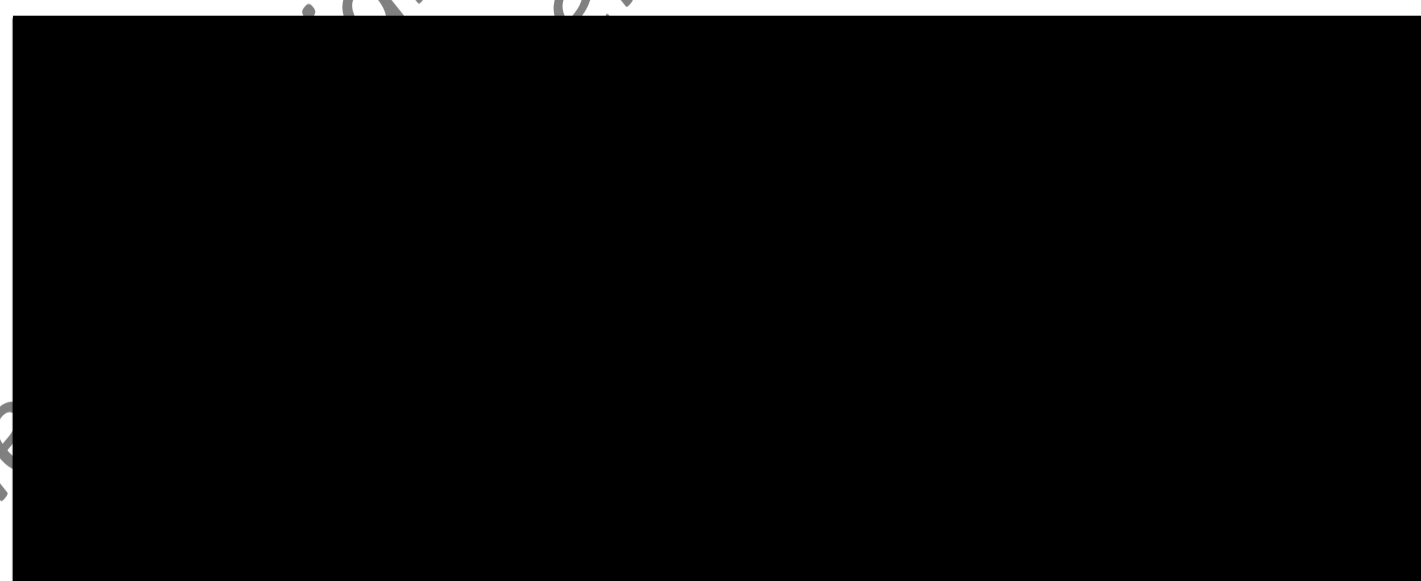
Delivered to: Bayer AG  
Institute for Environmental Biology  
Laboratory for non-target arthropods  
Internal laboratory no. 219

Date of reception: 13.4.2000

Contract laboratory: GAB/ Biotechnologie, Niefern-Öschelbrunn

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

Leverkusen, 21.6.00



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