



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

[Redacted]

**hive bees
(≤ 5 days)**

Date

09/06/2000

Testing facility

Arbeitsgemeinschaft

GAB Biotechnologie GmbH &

IFU Umweltanalytik GmbH

Eutingen Str. 24

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Germany

Sponsor

Bayer AG

Geschäftsbereich Pflanzenschutz

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Germany



20001148/01-BLEU 2. / MO-02-008333

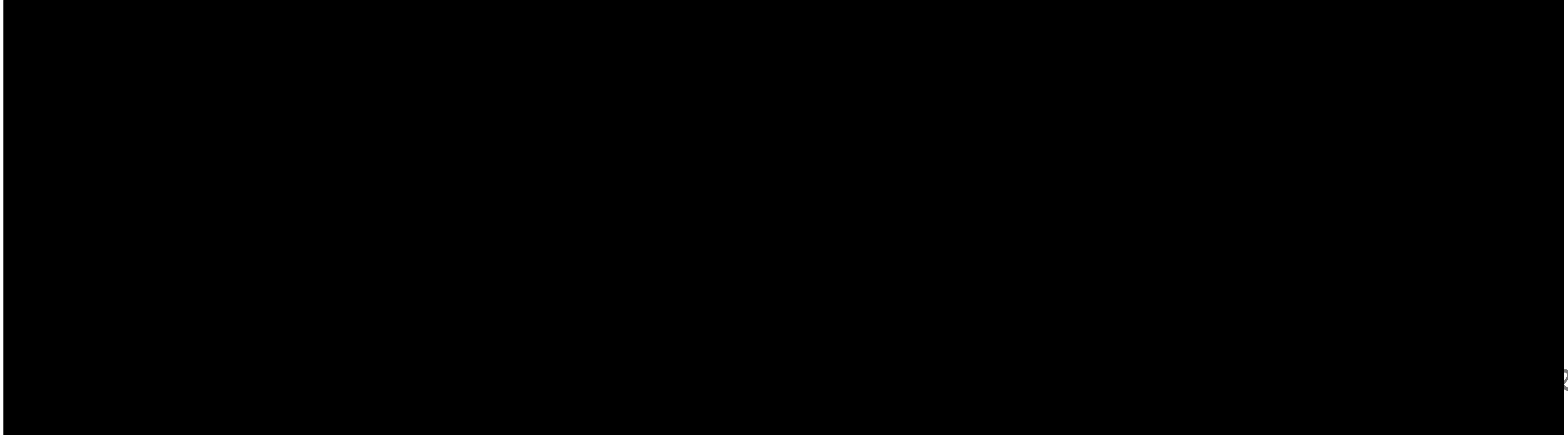
Study Identification Code

Test substance: Substance B

Study code: 20001148/01-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 B. 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.

The mortality in the Substance B treatment groups rose up to 8 %, observed in the treatment fed with the lowest concentrated test substance solution of 0.1 µg/L which corresponded to an actual intake of 0.04458 ng/bee after ten days.

No mortality occurred in the treatment group fed with the highest concentrated test substance solution (10 µg/L) of Substance B (actual intake: 4.316 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 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 *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, 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 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 B 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 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	-	0.5512	-	0
Substance B	0.1	0.5215	0.04458	8
	1	0.5314	0.45420	6
	10	0.5050	4.31623	0

*Weight of sucrose solution 1.17 mg/ml

The mortality in the treatment groups with Substance B rose up to 8 %, observed in the treatment fed with the lowest concentrated test substance solution of 0.1 µg/L which corresponded to an actual intake of 0.04457 ng/bee after ten days.

No mortality occurred in the treatment group fed with the highest concentrated test substance solutions (10 µg/L) of Substance B (actual intake: 4.31623 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

	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]
Date	10/05/2000	11/05/2000			12/05/2000			13/05/2000		
Control	7,520	7,098	0,422		6,593	0,505		6,038	0,555	
	7,513	7,119	0,394		6,843	0,276		6,531	0,312	
	7,489	6,911	0,578		6,332	0,579		5,806	0,526	
	7,508	6,987	0,521		6,491	0,496		5,914	0,577	
	7,687	7,258	0,429	0,469	6,769	0,489	0,469	6,443	0,326	0,469

	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]
Date	13/05/2000	14/05/2000			16/05/2000		
Control	7,484	6,952	0,735		5,426	1,526	
	7,524	6,983	0,704		5,688	1,295	
	7,517	7,031	0,656		5,478	1,553	
	7,491	7,132	0,555		6,002	1,130	
	7,480	7,029	0,658	0,662	5,712	1,317	1,364

	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]
Date	16/05/2000	18/05/2000		
Control	7,683	6,660	1,027	
	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

	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]
Date	18/05/2000	20/05/2000		
Control	7,535	5,847	1,840	
	7,568	6,974	0,713	
	7,628	5,783	1,904	
	7,715	6,315	1,379	
	7,600	6,725	0,962	1,358



GAB Calculation Sheet for Honey Bee Laboratory Tests (EPPO)
 Calculation of the intake of test substance
 Study code 20001148/01-BLEU
 Test substance Substance B
 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 [µg/bee]	Sum intake of test substance [µg/bee]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Average Intake [µg/bee]	Sum intake of test substance [µg/bee]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Average Intake [µg/bee]	Sum intake of test substance [µg/bee]
Date	10/05/2000	11/05/2000					12/05/2000					13/05/2000				
Concentration	7.481	6.843	0.638				6.092	0.751				5.466	0.626			
0,1	7.521	6.979	0.542				6.475	0.504				6.073	0.402			
µg/L	7.522	6.883	0.639				6.487	0.396				5.993	0.519			
	7.487	6.980	0.497				6.299	0.591				5.974	0.225			
	7.609	6.706	0.903	0.649	0.000055470	0.000055470	6.204	0.496	0.548	0.000046838	0.0000102308	5.579	0.625	0.518	0.000044274	0.0000146581
Concentration	7.862	7.036	0.826				6.605	0.431				6.167	0.438			
10	7.870	7.181	0.429				6.684	0.497				6.167	0.511			
µg/L	7.909	6.970	0.639				6.610	0.360				6.182	0.411			
	7.375	7.045	0.530				6.746	0.299				6.493	0.359			
	7.585	7.245	0.340	0.513	0.000043862	0.000043862	6.745	0.500	0.417	0.000036384	0.0000802145	6.273	0.472	0.426	0.000031533	0.0001173679
Concentration	7.265	6.716	0.549				6.347	0.369				5.946	0.401			
10	7.558	6.964	0.594				6.600	0.364				6.078	0.520			
µg/L	7.597	6.676	0.921				6.207	0.469				5.688	0.619			
	7.603	7.140	0.463				6.901	0.239				6.449	0.452			
	7.559	7.617	0.542	0.614	0.0005247863	0.0005247863	6.562	0.455	0.379	0.0003239216	0.0000487179	5.969	0.593	0.490	0.0004247863	0.0012735043

	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Average Intake [µg/bee]	Sum intake of test substance [µg/bee]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Average Intake [µg/bee]	Sum intake of test substance [µg/bee]
Date	13/05/2000	14/05/2000					15/05/2000				
Concentration	7.512	6.801	0.711				5.986	1.215			
0,1	7.495	7.084	0.411				6.080	1.004			
µg/L	7.417	6.879	0.538				5.889	0.990			
	7.504	6.922	0.582				5.861	1.061			
	7.506	6.895	0.611	0.561	0.000048927	0.0000195508	6.214	0.681	0.990	0.0000981416	0.000029549
Concentration	7.498	6.956	0.539				5.686	1.273			
10	7.499	6.687	0.812				5.949	1.338			
µg/L	7.482	7.064	0.418				6.610	1.254			
	7.452	7.146	0.306				6.394	0.752			
	7.433	7.026	0.404	0.496	0.0000432583	0.0001606262	6.684	1.145	0.92	0.0001004710	0.0002610972
Concentration	7.502	6.883	0.619				5.664	1.216			
10	7.483	7.180	0.323				6.155	1.065			
µg/L	7.451	6.868	0.583				5.782	0.866			
	7.473	7.139	0.334				6.300	0.839			
	7.445	6.836	0.609	0.494	0.0004222222	0.0016957265	5.371	1.465	0.123	0.000952828	0.002655555

	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Average Intake [µg/bee]	Sum intake of test substance [µg/bee]
Date	16/05/2000	18/05/2000				
Concentration	7.630	6.628	1.002			
0,1	7.621	6.759	0.862			
µg/L	7.671	6.619	1.052			
	7.773	6.724	1.049			
	7.647	7.034	0.613	0.916	0.000085098	0.000038748
Concentration	7.645	6.416	1.229			
10	7.608	6.812	0.896			
µg/L	7.738	6.675	1.063			
	7.582	6.773	0.816			
	7.643	6.326	1.319	1.084	0.0000945404	0.000656375
Concentration	7.732	6.920	0.812			
10	7.686	6.773	0.913			
µg/L	7.653	6.744	0.909			
	7.611	6.985	0.626			
	7.747	6.503	1.244	0.901	0.0007700855	0.0024258410

	Weight before feeding [g]	Weight after feeding [g]	Intake [g]	Average Intake [g]	Average Intake [µg/bee]	Sum intake of test substance [µg/bee]
Date	19/05/2000	20/05/2000				
Concentration	7.533	6.506	1.027			
0,1	7.531	6.512	1.019			
µg/L	7.534	6.511	1.023			
	7.574	6.576	0.998			
	7.596	7.062	0.534	0.970	0.000077016	0.0000445284
Concentration	7.534	6.663	0.871			
10	7.571	6.659	0.912			
µg/L	7.569	6.622	0.947			
	7.471	6.879	0.602			
	7.421	6.251	1.170	0.989	0.000091044	0.000494009
Concentration	7.500	6.784	0.716			
10	7.470	6.724	0.746			
µg/L	7.462	6.732	0.770			
	7.521	6.803	0.718			
	7.562	6.803	0.759	0.978	0.000090961	0.000470239

Substance B

Final Report

20001148/01-BLEU



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, Niefen-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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