Repeat Test

Substance C

Feeding Test on the
Honeybee *Apis mellifera* (Hymenoptera, Apidae), NON-GLP

according to the Guideline No. 170
of the European and Mediterranean Plant Protection
Organisation (EPPO)

Sponsor
Bayer AG
Geschäftsbereich Pflanzenschutz Entwicklung
Landwirtschaftszentrum Monheim Institut für Ökobiologie
[redacted]
Alfred-Nobel-Str. 50
D-51368 Leverkusen

Author
[redacted]

Test Facility
DR. U. NOACK-LABORATORIUM
FÜR ANGEWANDTE BIOLOGIE
Käthe-Paulus-Str. 1
D-31157 Sarstedt

Laboratory Project ID
Project-No. 000706BK
Study-No. IBA7242N

IBA7242N / MO-02-008338

Date
1 [illegible] Aug. 2006

Date
1 [illegible] Aug. 2006

Table of Content and Tables

Content

		Page
1	Summary	3
2	Method	4
3	Quality Criterion	6
4	Literature / References	7
5	Biological Data	7
5.1	Results of Worker Bees	7

Tables

Table 1	Mortality Values of Substance C, Worker Bees	3
Table 2:	Food Uptake of Test Item and Control (Worker Bees)	7
Table 3:	Mortality and Behaviour of Control and Substance C (Worker Bees)	10

1 Summary

Report: [redacted] (2000): Substance C,
Feeding Test on the Honeybee *Apis mellifera*, NON-GLP.
Source: DR. U. NOACK-LABORATORIUM FÜR ANGEWANDTE BIOLOGIE, unpublished report
Study No: IBA7242N, Project No: 000706BK

Guidelines: EPPO No. 170
Deviations: no major deviations

Material and methods: A feeding test was conducted over 10 days with the test item Substance C with the test concentrations of 0.1, 1.0, 10 ppb. Young worker bees (*Apis mellifera*) were kept in ventilated stainless steel cages, in 3 replicates of 10 individuals for each treatment. Control item was 50 % aqueous sucrose solution.

Mortality was assessed after 2, 4, 6, 8 and 10 days exposure period. The control and test item solutions were exchanged every 2 days and the ingested test item amount was calculated.

Dates of work: July 11 to 21, 2000

Findings: summarized in Table 1:

Table 1: **Mortality Values of Substance C, Worker Bees**

Test item concentration [ppb]	Ingested test item amount [ng a.i./bee]	Mortality [%] after 10 days	Corrected mortality [%] after 10 days
Control	-	7	-
0.1	0,059	10	3
1.0	0,551	7	0
10	5,801	7	0

2 Method

TEST GUIDELINES

Guideline No. 170 of the European and Mediterranean Plant Protection Organisation (EPPO), "Guideline on test methods for evaluating the side-effects of plant protection products on honeybees" (1992).

TYPE AND PURPOSE OF THE STUDY

The study was conducted in order to determine the effects of the test item on the Western honeybee, *Apis mellifera* under laboratory conditions, oral toxicity, long time feeding (10 days).

TEST SYSTEM

Apis mellifera L. (Hymenoptera, Apidae), subspecies *A. m. carnica* POLLM.

Reason for the selection of the test system

The honeybees are the most effective pollinator on a worldwide scale and may also be considered representatives of insect pollinators in general.

Source

Queen-right, healthy colony of the test facility, not used for honey harvest during the use of bees for the study.

Collection

One age stages of bees was used for the study.
Young worker bees were collected from honey frames by brushing them off. The age of the bees was ca. 12 – 17 days (DIEMER 1986).

Transport / Feeding

The bees were transferred immediately to the laboratory on the day before application. They were acclimatised to test conditions in a gauze-covered plastic (PP) bucket and allowed to feed ad libitum (50 % sucrose solution) for at least 1.

TEST ITEM

Substance C

Test concentrations

0.1, 1, 10 ppb tested with young worker bees.

Preparation of solutions was done at the beginning of the study.
Storage 4 °C dark.

CONTROL

Control item was 50 % aqueous sucrose solution.

TEST METHOD

Replicates

Each concentration of the Substance C and the controls were tested with three replicates with ten young worker bees. The bees were distributed randomised to the test cages.

Test vessels

Stainless steel cages, size 102 x 56 x 86 mm with holes punched in the bottom plate and a glass plate at front. The cages were fitted with two holes on the top, used as bee inlet and for placement of the feeding tube.

Application

In the oral application mode, the test item stock solutions were dissolved in a 50 % aqueous sucrose solution prepared with tap water, which was applied to groups of ten bees, which shared it equally among themselves due to their reciprocal social feeding behaviour termed "trophallaxis". Every 2 days new test item solution was given to the bees. The ingested test item solution was documented.

Climatic conditions

The study was carried out under stable climatic conditions:
(Documentation with a thermohygrograph)

	Temperature [°C]	Relative humidity [%]	Photoperiod
nominal	25 ± 2	40 - 80	Continuous dark
actual	23 - 25	59 – 80	Continuous dark

TYPE AND FREQUENCY OF MEASUREMENTS AND OBSERVATIONS

For the oral application mode the up taken test item amount was determined after 2, 4, 6, 8 and 10 days.
Mortality and abnormal behaviour was determined at least after 2, 4, 6, 8, 10 days.

COURSE OF THE STUDY

	• Preparation of sucrose solutions for oral application on day 0. • Labelling of feeding tubes for test item solutions and cages • Starvation: None.
Start	Adaptation to test conditions for ca. 1 h. Groups of ten bees were impartially transferred to the test cages. • Preparation of the test solutions. Storage at 4 °C. • Weighing of feeding tubes with amount of test item and insertion in test cages (start of application).
Day 2, 4, 6, 8, 10:	Assessment of behaviour and mortality. Exchange of feeding tubes. Weighing of feeding tubes.
End of test	Day 10
Correction of mortality rates	Mortality rates of test item were corrected against control according to Formula 1 (SCHNEIDER-ORELLI 1947) **M = [(Mt - Mc) / (100 - Mc)] x 100** (1) M = Corrected mortality [%] Mt = percentage mortality of the treated group; Mc = percentage mortality of the control group

3 Quality Criterion

- Mortality in the control should not exceed 15 % after 10 days.
  This study: Percentage mortality in the control was 7 % for worker bees.

Since the validity criterion is fulfilled for worker bees, the test part is assumed to be valid.

4 Literature / References

(1) European and Mediterranean Plant Protection Organisation (EPPO): Guideline on test methods for evaluating the side-effects of plant protection products on honeybees. Bulletin OEPP EPPO Bulletin 22, 203 - 215 (1992) = Guideline No. 170

(2) DIEMER, I. (1986): Bienen. Kosmos Gesellschaft der Naturfreunde. Franck'sche Verlagsbuchhandlung, Stuttgart.

(3) SCHNEIDER-ORELLI, O. (1947): Entomologisches Praktikum, Einführung in die land- und forstwirtschaftliche Insektenkunde. Verlag H. R. Sauerländer & Co. Aarau.

5 Biological Data

5.1 Results of Worker Bees

Table 2: **Food Uptake of Test Item and Control (Worker Bees)**

Nominal concentration [ppb]	Rp.	Feeding interval	Weight of feeding tubes before feeding [g]	Weight of feeding tubes after feeding	Food uptake [g]	Food uptake [g]	Mean food uptake (10 bees) [g]	Real concentration [ng a.i./bee]
Control	1	1	4,5972	3,9068	0,6904	3,9523	5,9415	-
		2	4,5963	3,7414	0,8549			
		3	4,7708	3,8417	0,9291			
		4	4,7343	3,8896	08447			
		5	4,7397	4,1065	0,6332			
	2	1	4,5705	3,6496	0,9209	6,6753		
		2	4,6518	3,5619	1,0899			
		3	4,6572	2,8844	1,7728			
		4	4,6676	3,1338	1,5338			
		5	4,6756	3,3177	1,3579			
	3	1	4,6305	3,3951	1,2354	7,1968		
		2	4,6564	3,4107	1,2457			
		3	4,6580	2,8490	1,809			
		4	4,6604	3,0312	1,6292			
		5	4,6808	3,4033	1,2775			

Rp. = Replicate no.

Table 2 (continued): **Food Uptake of Test Item and Control (Worker Bees)**

Nominal concentration [ppb]	Rp.	Feeding interval	Weight of feeding tubes before feeding [g]	Weight of feeding tubes after feeding	Food uptake [g]	Food uptake [g]	Mean food uptake (10 bees) [g]	Real concentration [ng a.i./bee]
0.1	1	1	4,5176	3,2088	1,3088	6,424	5,8692	0,059
		2	4,5863	3,2247	1,3616			
		3	4,5685	2,9151	1,6534			
		4	4,548	3,3297	1,2183			
		5	4,6514	3,7695	0,8819			
	2	1	4,6163	3,9177	0,6986	4,6854		
		2	4,6843	3,863	0,8213			
		3	4,6416	3,5255	1,1161			
		4	4,6601	3,6211	1,039			
		5	4,6585	3,6481	1,0104			
	3	1	4,5996	3,5282	1,0714	6,4983		
		2	4,6111	3,4278	1,1833			
		3	4,6248	3,1064	1,5184			
		4	4,6142	3,2678	1,3464			
		5	4,5845	3,2057	1,3788			
1.0	1	1	4,5022	3,0785	1,4237	5,1914	5,5073	0,551
		2	4,6646	3,573	1,0916			
		3	4,6582	3,6261	1,0321			
		4	4,6464	3,7499	0,8965			
		5	4,6632	3,9157	0,7475			
	2	1	4,6453	3,3449	1,3004	5,8139		
		2	4,6545	3,5411	1,1134			
		3	4,6721	3,2058	1,4663			
		4	4,6707	3,4128	1,2579			
		5	4,6785	4,0026	0,6759			
	3	1	4,6085	3,6543	0,9542	5,5165		
		2	4,6234	3,6356	0,9878			
		3	4,6455	3,1718	1,4737			
		4	4,6593	3,4538	1,2055			
		5	4,6453	3,75	0,8953			

Rp. = Replicate no.

Table 2 (continued): **Food Uptake of Test Item and Control (Worker Bees)**

Nominal concentration [ppb]	Rp.	Feeding interval	Weight of feeding tubes before feeding [g]	Weight of feeding tubes after feeding [g]	Food uptake [g]	Food uptake [g]	Mean food uptake (10 bees) [g]	Real concentration [ng a.i./bee]
10	1	1	4,6248	3,6934	0,9314	5,8447	5,8014	5,801
		2	4,633	3,8172	0,8158			
		3	4,6468	3,4504	1,1964			
		4	4,6461	3,3834	1,2627			
		5	4,6442	3,0058	1,6384			
	2	1	4,5485	3,5563	0,9922	5,6457		
		2	4,6164	3,903	0,7134			
		3	4,6514	3,677	0,9744			
		4	4,6209	3,1186	1,5023			
		5	4,6791	3,2157	1,4634			
	3	1	4,4022	3,5517	0,8505	5,9137		
		2	4,7356	3,767	0,9686			
		3	4,7479	3,4995	1,2484			
		4	4,7418	3,2622	1,4796			
		5	4,7362	3,3696	1,3666			

Rp. = Replicate no.

Table 3: **Mortality and Behaviour of Control and Substance C (Worker Bees)**

Concentration [ppb]	Replicate	Effect	Time [d] 2	4	6	8	10
Control	1	N	10/10	10/10	10/10	9/10	9/10
		M	0/10	0/10	0/10	1/10	1/10
	2	N	10/10	10/10	10/10	10/10	10/10
	3	N	9/10	9/10	9/10	9/10	9/10
		M	1/10	1/10	1/10	1/10	1/10
	Sum mortality [%]	**M**	**3**	**3**	**3**	**7**	**7**
0.1	1	N	10/10	10/10	8/10	8/10	8/10
		L	0/10	0/10	1/10	1/10	0/10
		M	0/10	0/10	1/10	1/10	2/10
	2	N	10/10	10/10	10/10	10/10	9/10
		L	0/10	0/10	0/10	0/10	0/10
		M	0/10	0/10	0/10	0/10	1/10
	3	N	10/10	10/10	10/10	10/10	10/10
		L	0/10	0/10	0/10	0/10	0/10
		M	0/10	0/10	0/10	0/10	0/10
	Sum mortality [%]	**M**	**0**	**0**	**3**	**3**	**10**
1.0	1	N	10/10	10/10	9/10	9/10	9/10
		L	0/10	0/10	0/10	0/10	0/10
		M	0/10	0/10	1/10	1/10	1/10
	2	N	10/10	9/10	9/10	9/10	9/10
		L	0/10	1/10	1/10	0/10	0/10
		M	0/10	0/10	0/10	1/10	1/10
	3	N	10/10	9/10	9/10	9/10	9/10
		L	0/10	1/10	1/10	1/10	1/10
		M	0/10	0/10	0/10	0/10	0/10
	Sum mortality [%]	**M**	**0**	**0**	**3**	**7**	**7**
10	1	N	10/10	10/10	10/10	10/10	10/10
		L	0/10	0/10	0/10	0/10	0/10
		M	0/10	0/10	0/10	0/10	0/10
	2	N	9/10	9/10	9/10	9/10	9/10
		L	0/10	0/10	0/10	0/10	0/10
		M	1/10	1/10	1/10	1/10	1/10
	3	N	10/10	10/10	10/10	10/10	8/10
		L	0/10	0/10	0/10	0/10	1/10
		M	0/10	0/10	0/10	0/10	1/10
	Sum mortality [%]	**M**	**3**	**3**	**3**	**3**	**7**

M = Dead
L = Slow motions/problems concerning coordination
N = Normal behaviour

16.8.00
Date

[redacted]

16.8.00
Date

[redacted]