Substance A

Acute Effects 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

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Laboratory Project ID

Project-No.	000420BK
Study-No.	IBA7240N

Date

19. Juni 2000

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1 Summary

Report: [redacted] Substance A,
Acute Effects on the Honeybee *Apis mellifera*, NON-GLP.
Source: DR. U. NOACK-LABORATORIUM FÜR ANGEWANDTE BIOLOGIE, unpublished report
Study No: IBA7240N, Project No: 000420BK

Guidelines: EPPO No. 170
Deviations: no major deviations

Material and methods: The test item Substance A was applied at nominal concentrations of 1, 3, 9, 27, 81 ng a.i. per bee for oral application and of 40, 56, 78, 110, 154 ng a.i. per bee (dissolved in acetone) for contact application under laboratory conditions.
Control item in oral application mode was 50 % aqueous sucrose solution with same content of acetone as test item. For the contact application acetone was used as control.

Apis mellifera (only worker bees), were kept in ventilated stainless steel cages, in 3 replicates of 10 individuals for each treatment. Mortality was assessed after a 4, 24 and 48 h exposure period and additional after 96 h in oral application mode.

Dates of work: May 05 to 08, 2000

Findings: summarized in Tables 1-2.

Table 1 **Oral Application Mode: Mortality Values of Substance A**

Concentration [ng a.i./Biene]		Mortality [%]
Nominal	Real*	48 [h]
Control	0.00	0
1	0.94	0
3	2.81	3
9	7.01	0
27	17.80	7
81	34.70	17
LD_{50} [ng a.i./bee] after 48 h		> 34.70

* Application rate based on actual ingestion of the test item

Table 2 **Contact Application Mode: Mortality Values of Substance A**

Concentration [ng a.i./Biene]	Mortality [%] after 48 h
Control	0
40	43
56	63
78	73
110	90
154	87
LD_{50} [ng a.i./bee]after 48 h	42.92
Confidence interval	34.64 – 53.19

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 and contact toxicity.

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

Young worker bees were collected from the frame by brushing them off.

Transport / Feeding

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

TEST ITEM

Substance A

Test concentrations

Oral : 1, 3, 9, 27, 81 ng / bee
Topical : 40, 56, 78, 110, 154 ng / bee

Preparation of stock solutions with acetone for each test item concentration (oral and contact). Oral: 0.1 mL stock solution was added to 3.97 mL sucrose solution to prepare test item solutions.

CONTROL

Control item in oral application mode was 50 % aqueous sucrose solution with same content of acetone as test item. For the contact application acetone was used.

TEST METHOD

Replicates

Each variant was tested with three replicates.

Number and age of the bees/ replicate

10 young impartially selected adult bees - but not from brood nest - per cage.

Test vessels

Stainless steel cages, size 102 x 56 x 86 mm with holes punched in the bottom plate and a glassplate 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 was dissolved in 0.2 mL of 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".

For the contact application mode, the amount to be applied to each individual bee was dissolved in acetone. The test item solutions (5 µL) was applied to the thorax of each bee individually with a micro piston pipette. The bees were paralysed with for 25 sec with CO_2.

Climatic conditions

The study was carried out under stable climatic conditions:

	Temperature [°C]	Relative humidity [%]	Photoperiod
nominal	25 ± 2	40 - 80	Continuous dark
actual	25 - 26	60 - 84	Continuous dark

(Documentation with a thermohygrograph)

Feeding

After application and during the study the bees were fed ad libitum with 50 % sucrose solution (household quality).

TYPE AND FREQUENCY OF MEASUREMENTS AND OBSERVATIONS

For the oral application mode the uptaken test item amount was determined after 3 h by weight.
Mortality and abnormal behaviour in oral and contact application mode were determined after 4, 24 and 48 h and additional in oral application mode after 96 h.

COURSE OF THE STUDY

- Preparation of sucrose solutions for oral application on day 0.
- also labelling of feeding tubes for test item solutions and cages
- Filling of tubes with 50 % sucrose solution for ad libitum feeding

Start	Adaptation to test conditions for ca. 1 h Groups of ten bees were impartionally transferred to the test cages.
	Oral test mode: • Starvation of the bees for up to two hours prior to oral application. • Preparation of the test solutions • Weighing of feeding tubes with amount of test item and insertion in test cages (start of application). • after 3 h: exchange of feeding tubes against feeding tubes with 2 mL 50 % sugar solution. • Reweighing of feeding tubes for documentation of food.
4, 24, 48 and 96 h (only oral application mode)	Assessment of behaviour and mortality. Exchange of feeding tube after 48 h.
End of test	After 48 h in contact application mode, after 96 h in oral application mode.

EVALUATION

Correction of mortality rates	Mortality rates of test item concentrations were not corrected against control as control mortality was 0 %.

LD_{50} determination	Oral application mode: LD_{50}-value of the test item was determined directly from the test results. There was no calculation. Contact application mode: LD_{50}-value was calculated by probit analysis. Probit values according to WEBER (1986). Calculation of the confidence intervals for LD_{50} were carried out using standard procedures according to BREITIG & TÜMPLING (1982).

3 Quality Criterion

- Mortality in the control should not exceed 15 % after 48 h.
  This study: Percentage mortality in the control was
  0 % in oral and in contact application mode.

Since the validity criterion is fulfilled, the test is assumed to be valid.

4 Literature / References

(1) BREITIG, G., TÜMPLING, W. (editors, 1982): Methoden der Wasseruntersuchungen Bd.II. VEB GUSTAV FISCHER VERLAG, Jena

(2) 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

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

(4) WEBER, E. (1982): Grundriß der biologischen Statistik, 9. Auflage (1986).

Testing Procedures for Pesticides with Non-Target Arthropods. ISBN 0 9522535 2 6.

5 Biological Data

Table 3: **Oral Application Mode: Food Uptake of Test Item and Control after 3 h**

Nominal concentration [ng a.i./bee	Rp.	Weight of feeding tubes before feeding [g]	Weight of feeding tubes after feeding [g]	Food uptake [g]	Food uptake mean ± SD [g]	Food uptake [%]#	Actual intake [ng a.i./bee]
Control	1	1.5840	1.3858	0.1982	0.2141 ± 0.0144	87.39	0.00
	2	1.5453	1.3191	0.2262			
	3	1.5795	1.3616	0.2179			
1	1	1.5575	1.3237	0.2338	0.2296 ± 0.0037	93.70	0.937
	2	1.5210	1.2938	0.2272			
	3	1.6135	1.3858	0.2277			
3	1	1.5529	1.3304	0.2225	0.2293 ± 0.0069	93.58	2.81
	2	1.5846	1.3555	0.2291			
	3	1.6124	1.3762	0.2362			
9	1	1.5474	1.3749	0.1725	0.1908 ± 0.0166	77.89	7.01
	2	1.5922	1.3971	0.1951			
	3	1.5731	1.3682	0.2049			
27	1	1.5846	1.4376	0.1470	0.1615 ± 0.0295	65.90	17.80
	2	1.5910	1.3956	0.1954			
	3	1.5934	1.4514	0.1420			
81	1	1.5648	1.4697	0.0951	0.1049 ± 0.0342	42.82	34.70
	2	1.5485	1.4718	0.0767			
	3	1.5942	1.4513	0.1429			

Rp. = Replicate no.
*) Statistical analysis carried out using t-test with vehicle control
#) Notice: 100 % food uptake =
0.2 mL = 0.245 g (depending on density (1.227 g/mL) of 50 % sugar solution)

Table 4: **Oral Application Mode: Mortality and Behaviour of Control and Substance A**

Actual intake [ng a.i./bee]	Replicate	Effect	Time [h] 4	24	48	96
Control	1	N	10/10	10/10	10/10	10/10
	2	N	10/10	10/10	10/10	10/10
	3	N	10/10	10/10	10/10	10/10
	Sum mortality [%]	**M**	**0**	**0**	**0**	**0**
0.94	1	N	10/10	10/10	10/10	10/10
	2	N	10/10	10/10	10/10	10/10
	3	N	10/10	10/10	10/10	10/10
	Sum mortality [%]	**M**	**0**	**0**	**0**	**0**
2.81	1	N	10/10	10/10	10/10	10/10
	2	N	10/10	10/10	10/10	10/10
	3	N	10/10	9/10	9/10	9/10
		M	0/10	1/10	1/10	1/10
	Sum mortality [%]	**M**	**0**	**3**	**3**	**3**
7.01	1	N	10/10	10/10	10/10	9/10
		M	0/10	0/10	0/10	1/10
	2	N	10/10	10/10	10/10	10/10
	3	N	10/10	10/10	10/10	10/10
	Sum mortality [%]	**M**	**0**	**0**	**0**	**3**
17.80	1	N	10/10	10/10	9/10	9/10
		M	0/10	0/10	1/10	1/10
	2	N	10/10	10/10	10/10	10/10
	3	N	10/10	9/10	9/10	9/10
		M	0/10	1/10	1/10	1/10
	Sum mortality [%]	**M**	**0**	**3**	**7**	**7**
34.70	1	N	10/10	9/10	9/10	7/10
		R	0/10	1/10	1/10	0/10
		M	0/10	0/10	0/10	3/10
	2	N	10/10	9/10	8/10	8/10
		M	0/10	1/10	2/10	2/10
	3	N	10/10	6/10	7/10	4/10
		R	0/10	2/10	0/10	0/10
		M	0/10	2/10	3/10	6/10
	Sum mortality [%]	**M**	**0**	**10**	**17**	**37**

M = Dead
R = Lying on its back, problems with standing up
N = Normal behaviour

Report
Substance A
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Project-No. 000420BK
Study No. IBA7240N

Table 5: **Contact Application Mode: Mortality and Behaviour of Control and Substance A**

Concentration [ng a.i./bee]	Replicate	Effect	Time [h] 4	24	48
Control	1	N	10/10	10/10	10/10
	2	N	10/10	10/10	10/10
	3	N	10/10	10/10	10/10
	Sum mortality [%]	**M**	**0**	**0**	**0**
40	1	N	10/10	0/10	0/10
		R	0/10	3/10	0/10
		L	0/10	7/10	6/10
		M	0/10	0/10	4/10
	2	N	10/10	0/10	3/10
		R	0/10	7/10	1/10
		L	0/10	3/10	0/10
		M	0/10	0/10	6/10
	3	N	10/10	1/10	5/10
		R	0/10	2/10	2/10
		L	0/10	7/10	0/10
		M	0/10	0/10	3/10
	Sum mortality [%]	**M**	**0**	**0**	**43**
56	1	N	10/10	0/10	0/10
		R	0/10	5/10	0/10
		L	0/10	5/10	3/10
		M	0/10	0/10	7/10
	2	N	10/10	0/10	0/10
		R	0/10	6/10	0/10
		L	0/10	4/10	4/10
		M	0/10	0/10	6/10
	3	N	5/10	0/10	0/10
		R	3/10	10/10	2/10
		L	2/10	0/10	2/10
		M	0/10	0/10	6/10
	Sum mortality [%]	**M**	**0**	**0**	**63**

M = Dead
R = Lying on its back, problems with standing up
L = Slow motions/problems concerning coordination
N = Normal behaviour

Table 5 continued

Concentration [ng a.i./bee]	Replicate	Effect	Time [h] 4	24	48
78	1	N	9/10	0/10	0/10
		R	1/10	7/10	1/10
		L	0/10	3/10	2/10
		M	0/10	0/10	7/10
	2	N	9/10	0/10	0/10
		R	1/10	9/10	1/10
		L	0/10	1/10	0/10
		M	0/10	0/10	9/10
	3	N	8/10	0/10	0/10
		R	2/10	9/10	4/10
		L	0/10	1/10	0/10
		M	0/10	0/10	6/10
	Sum mortality [%]	**M**	**0**	**0**	**73**
110	1	N	7/10	0/10	0/10
		R	3/10	6/10	2/10
		L	0/10	2/10	0/10
		M	0/10	2/10	8/10
	2	N	8/10	0/10	0/10
		R	2/10	3/10	1/10
		L	0/10	0/10	0/10
		M	0/10	7/10	9/10
	3	N	6/10	0/10	0/10
		R	4/10	5/10	0/10
		L	0/10	0/10	0/10
		M	0/10	5/10	10/10
	Sum mortality [%]	**M**	**0**	**47**	**90**
154	1	N	6/10	0/10	0/10
		R	1/10	5/10	0/10
		L	3/10	0/10	0/10
		M	0/10	5/10	10/10
	2	N	5/10	0/10	1/10
		R	3/10	4/10	1/10
		L	2/10	1/10	0/10
		M	0/10	5/10	8/10
	3	N	6/10	0/10	0/10
		R	2/10	3/10	2/10
		L	2/10	3/10	0/10
		M	0/10	4/10	8/10
	Sum mortality [%]	**M**	**0**	**47**	**87**

M = Dead
R = Lying on its back, problems with standing up
L = Slow motions/problems concerning coordination
N = Normal behaviour

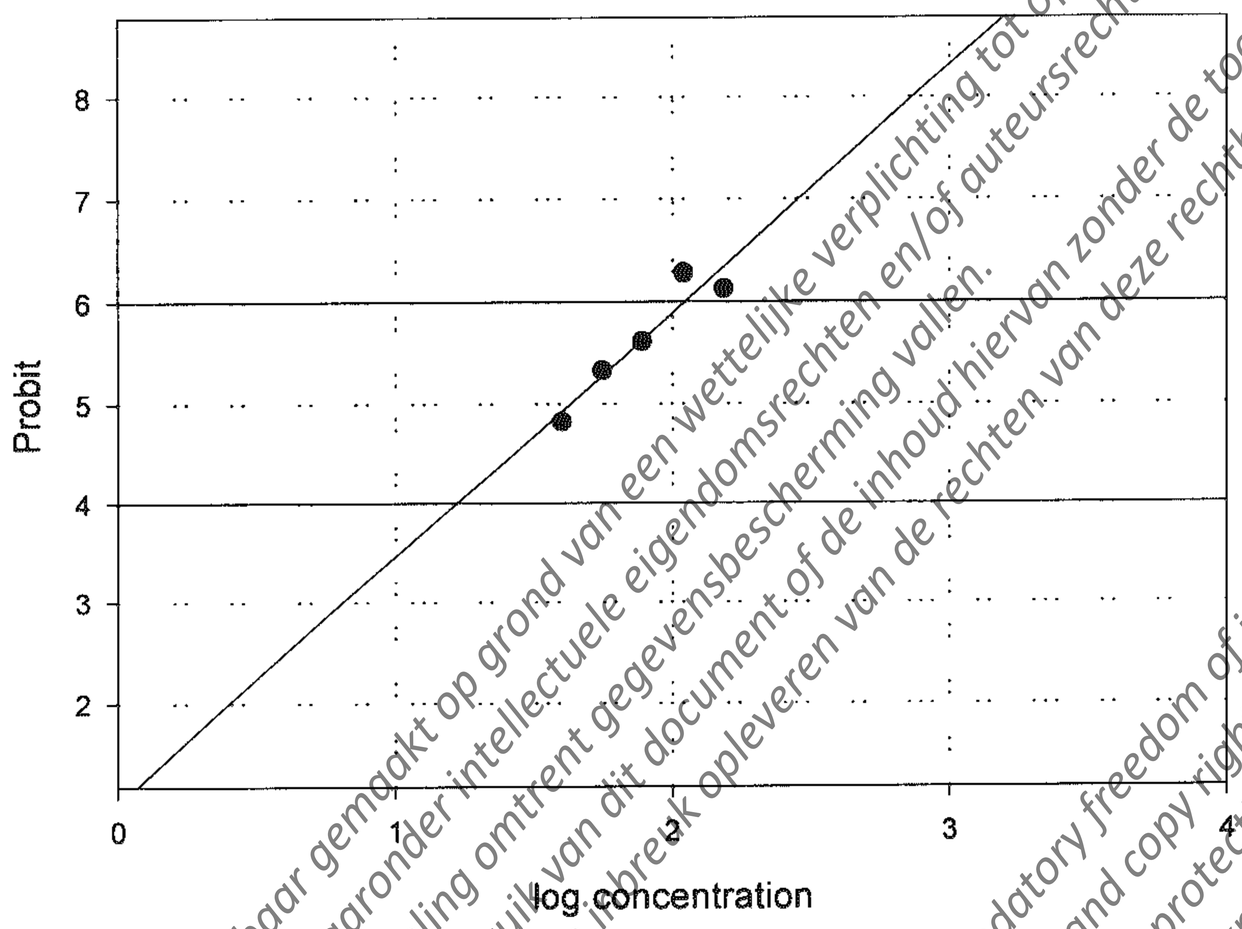

Figure 1: **Contact Application Mode: Dose Response Realationship of Mortality of Substance A**

19.06.2000
Date

[redacted]

Date

Amendment to Report No. IBA7240N

Identification of test substance

Code name in report:	Test substance A
Name of test substance:	NTN33893 (a.i.)
Origin of test substance:	Bayer AG, Leverkusen PF-Production
Specification	
Tox. no.:	5255
Article no.:	04145852
Batch no:	230924394
a.i. content:	98.6 %
Date of analysis:	30.3.2000
Expiry date:	30.10.2000
Delivered to:	Bayer AG Institute for Environmental Biology Laboratory for non-target arthropods Internal laboratory no. 218
Date of reception:	13.4.2000
Contract laboratory:	[redacted]
Date of delivery as substance A:	18.4.2000
Delivered amount:	1.13 g
Order no.:	347473k0

Leverkusen, 21.6.00

[redacted]