STUDY REPORT

Name and address of sponsor:	Bayer AG Landwirtschaftszentrum Monheim Institut fur Okobiologie 51368 Leverkusen Germany
Sponsor's Representative	Dr Jurgen Keppler
Name and address of test facility:	Environmental R&D Team Environmental Biology Group Central Science Laboratory Sand Hutton York N Yorks YO41 1LZ Tel 01904 462515, Fax 01904 462240
Study Director	Dr H M Thompson
Study dates:	
Initiation	27 April 2000
Experiment start	8 May 2000
Experiment end	21 May 2000
Study Completion	9 June 2000

Study Number HT0400c

Substance B:
Feeding Study with Honey Bees
(*Apis mellifera*).

HT0400c / MO-02-008306

1. Summary

Tests were carried out to determine the effect of feeding Substance B on mortality of adult honey bees (*Apis mellifera* L.) over a 10 day period. All doses and toxicity data for the test substance refer to Substance B as the active ingredient.

Five batches of bees, in groups of 10 bees, were offered 10, 1.0 and 0.1 ng/ ml Substance B in 50% w/v aqueous sucrose solution.

Mortality was assessed daily after dosing. Glass test feeders were removed and weighed and replaced with fresh feed each day.

Results indicated that the Substance B had no significant effect on mortality.

2. Introduction

This study was carried out on behalf of Bayer AG to establish the effect of feeding Substance B for 10 days on mortality of adult honey bees (*Apis mellifera* L.) in the laboratory.

The honey bee was chosen as the test organism, being representative of the pollinating insects which may be at risk if flowering crops are sprayed with pesticides. The effects of oral exposure are assessed in aqueous sucrose solution.

All doses and toxicity data for the test substance refer to Substance B as the active ingredient.

3. Materials and methods

3.1 Test substance

Substance B supplied by Bayer AG was used in this study. Each sample of the material tested in this study was uniquely labelled with Test Substance number NBU84. The test substance was stored, as recommended by the sponsor, at 4°C in the dark.

Substance B was dispersed in 50% aqueous sucrose for dosing and stored at 4°C until required.

Although there were no data on the stability of the test substance in solution the test substance was assumed to be stable under the conditions of this study.

3.2 Test bees

Test bees for all the tests in this study were adult worker bees (*Apis mellifera* L.) taken from a single colony (colony Thorpe 1) owned and maintained by the Central Science Laboratory's National Bee Unit for use in such tests.

3.3 General Method

Worker bees were collected from the hive by using a small amount of smoke, gently shaking them from the combs and transferring them (40-50 per cage) into cylindrical mesh cages.

In the laboratory the mesh cages were placed into the incubator (25 ± 1°C, 65 ± 5% relative humidity) before being used for the test. Immediately prior to treatment each mesh cage of bees was anaesthetised with carbon dioxide gas by placing the cage into a 2 litre beaker filled with CO_2.

A range of dose rates and a control were used, with five replicates of 10 bees per dose rate. During the test period the bees were kept in the dark (except during observations) in an incubator at 25 ± 1°C and 65 ± 5% relative humidity. Mortality

and sub-lethal effects were assessed at 24 hour intervals after the start of the test up to 10 days. The glass test feeders, containing any unconsumed portions of the doses, were removed and weighed daily and fresh test feed was supplied in pre-weighed glass feeders within the cages.

Sub-lethal effects were assessed according to pre-determined categories:
Knocked down (i.e. alive but immobile)
Stumbling (i.e. moving but in a poorly co-ordinated manner)

In this report "solution" is used to include material which may be suspended or dispersed. Homogeneity of such "solutions" of the Substance B were checked visually immediately before use. Solutions of the test doses were mixed well immediately prior to use and were homogenous for the purpose of administration.

Doses for the oral toxicity test (Table 1) were prepared from a stock solution of 1340 ng/ml Substance B in 50% w/v aqueous sucrose. The highest dose was prepared by diluting 0.746 ml stock to10 ml with 50% w/v aqueous sucrose and then preparing serial dilutions of this. Each group of 10 bees were offered up to 0.4 ml in each of two glass feeders in each test cage.

Control bees were given 50% w/v aqueous sucrose solution.

Table 1. Oral test dose levels

Dose group	Substance B ng /ml
1	10
2	1.0
3	0.1
Control	50% w/v aqueous sucrose

The bees were anaesthetised with carbon dioxide immediately before dosing and were gently tipped out onto filter paper and counted into the petri dish cage (drones were discarded). Each group of 10 bees was offered up to 0.8 ml of a given concentration (or controls as above), the dose being measured into two small, pre-weighed, glass feeders within the cage using a variable volume Gilson pipette.

After each 24 hour period the glass feeders were removed, weighed and replaced with fresh feed. The dose consumed was determined by comparison of the weight of the dose remaining in the glass feeders with the weight of a known volume of the test solutions.

4. Results

Substance B readily dissolved in 50% w/v aqueous sucrose at 1340 ng/ml to give a clear, colourless solution and remained in solution when further diluted in 50% w/v aqueous sucrose.

The mortality results for the oral test are listed in Table 2 for Substance B. Sub-lethal effects recorded are shown in Table 3.

TABLE 2. Results of oral dosing tests with Substance B

	dose	Number of bees dead (n=10)										dose taken
Cage	ng/ml	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	ml
105	10	1	1	1	2	2	2	2	2	2	3	6.94
106	10	0	0	0	0	0	1	2	2	2	2	6.61
107	10	1	1	1	1	1	7	9	9	9	9	5.58
108	10	0	0	0	4	4	4	4	4	4	4	6.16
109	10	1	1	1	1	1	3	4	4	4	4	6.71
110	1	0	0	0	0	0	0	0	0	0	2	7.10
111	1	0	0	0	2	3	3	3	3	3	3	6.43
112	1	0	0	0	3	3	3	3	3	3	3	6.33
113	1	0	1	1	1	1	3	3	3	3	3	6.63
114	1	0	0	1	3	3	3	3	6	6	6	6.35
115	0.1	1	1	2	3	3	3	4	5	5	5	6.96
116	0.1	0	0	0	0	0	1	1	6	6	6	6.73
117	0.1	0	0	1	2	2	2	2	2	3	3	7.07
118	0.1	0	0	1	4	4	4	4	4	4	4	6.16
119	0.1	0	0	0	0	2	4	4	4	4	5	7.14
120	0	0	0	0	0	0	3	3	3	3	3	6.73
121	0	1	0	5	4	4	4	4	7	7	7	6.16
122	0	0	0	3	4	4	7	7	7	7	7	6.46
123	0	0	0	3	4	4	3	4	4	4	4	6.77
124	0	1	2	2	3	3	3	3	3	3	4	6.99

TABLE 3. Sub-lethal effects observed in oral dosing tests with Substance B.

	Dose	Number of bees knocked down (K) or stumbling (S) (n=10)										Dose taken
Cage	ng/ml	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	ml
105	10	0	0	1K	0	0	0	0	0	0	0	6.94
106	10	0	0	0	0	0	1K	0	0	0	0	6.61
107	10	0	0	0	0	0	1S, 1K	0	0	0	0	5.58
108	10	0	0	1K	0	0	0	0	0	0	0	6.16
109	10	0	0	0	0	0	0	0	0	0	0	6.71
110	1	0	0	0	0	0	0	0	0	0	0	7.10
111	1	0	0	0	1K	0	0	0	0	0	0	6.43
112	1	0	0	2K	0	0	0	0	0	0	0	6.33
113	1	0	0	0	0	2K	0	0	0	0	0	6.63
114	1	0	0	0	0	0	0	0	0	0	0	6.35
115	0.1	0	0	0	0	0	0	0	0	0	0	6.96
116	0.1	0	0	1K	0	1K	0	0	0	0	0	6.73
117	0.1	0	0	1K	0	0	0	0	0	0	0	7.07
118	0.1	0	0	1K	0	0	0	0	0	0	0	6.16
119	0.1	0	0	0	0	2K	0	0	0	0	0	7.14
120	0	0	0	0	0	0	0	0	0	0	0	6.73
121	0	0	1K	0	0	0	0	0	0	0	0	6.16
122	0	0	0	0	1K	0	0	0	0	0	0	6.46
123	0	0	0	1K	0	1K	1K	0	0	0	0	6.77
124	0	1K	0	2K	0	0	0	0	0	0	0	6.99

Table 4. Oral toxicity of Substance B

Dose ng/ml	mean mortality (n=50) 1	2	3	4	5	6	7	8	9	10	Dose mls
10	1	1	1	2	2	3	4	4	4	4	6.40
1	0	0	0	2	2	2	2	3	3	3	6.57
0.1	0	0	1	2	2	3	3	4	4	5	6.81
0	0	0	3	3	3	4	4	5	5	5	6.62

Student's t test mortality compared to controls

Dose ng/ml	Day p= 1	2	3	4	5	6	7	8	9	10
10	0.2898	0.3405	0.0231	0.1055	0.1055	0.3269	0.5000	0.3566	0.3566	0.3469
1	0.0706	0.3333	0.0160	0.1255	0.1733	0.0706	0.0472	0.1048	0.1048	0.0878
0.1	0.2724	0.3333	0.0395	0.1563	0.2277	0.1255	0.1255	0.3051	0.3564	0.3469

6. Discussion and conclusions

No reduced intake of Substance B was observed even at the highest dose (10 ng/ml). The mortality was not significantly greater in bees offered Substance B treated feed to controls.

<u>Distribution</u>

Sponsor — Dr J Keppler (2 copies)
Study Director — Dr H M Thompson, CSL Environmental R&D

APPENDIX I

ACTUAL TIMETABLE FOR THE STUDY

ACUTE ORAL HONEYBEE TOXICITY TEST HT0400c

Day	Time (hours)	Date & Actual Time	Activity
-3	pm	08/05/00	Dispense test substance
0	-2½	12/05/00	Start bee collection
	-2	12/05/00	Stock prepared and dilutions started
	-1	12/05/00 13:45	Bees in incubator
	-1	12/05/00	Dilutions finished
	0	12/05/00 14:59	Orals dosed
1	+24	13/05/00 14:59	Assess, remove feeders, weigh and replace
2	+48	14/05/00 14:39	Assess, remove feeders, weigh and replace
3	+72	15/05/00 14:56	Assess, remove feeders, weigh and replace
4	+96	16/05/00 15:05	Assess, remove feeders, weigh and replace
5	+120	17/05/00 15:05	Assess, remove feeders, weigh and replace
6	+144	18/05/00 16:05	Assess, remove feeders, weigh and replace
7	+168	19/05/00 14:40	Assess, remove feeders, weigh and replace
8	+192	20/05/00 14:44	Assess, remove feeders, weigh and replace

9	+216	21/05/00 14:21	Assess, remove feeders, weigh and replace
10	+240	22/05/00 14:28	Assess, remove feeders, weigh and replace

STUDY REPORT AMENDMENT 1

Name and address of sponsor:	Bayer AG Landwirtschaftszentrum Monheim Institut fur Okobiologie 51368 Leverkusen Germany
Sponsor's Representative	Dr Jurgen Keppler
Name and address of test facility:	Environmental R&D Team Environmental Biology Group Central Science Laboratory Sand Hutton York N Yorks YO41 1LZ Tel 01904 462515, Fax 01904 462240
Study Director	Dr H M Thompson
Study dates:	
Initiation	27 April 2000
Experiment start	8 May 2000
Experiment end	21 May 2000
Study Completion	9 June 2000

Study Number HT0400c

Substance B:
Feeding Study with Honey Bees
(*Apis mellifera*).

Clarification
The bees used were fully developed nurse bees which had not just emerged, i.e. they did not still have hairs on their thorax and had fully extended wings. As guidance they were between 2 days and 2-3 weeks of age

Amendment to Report No. HT0400c

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:	Central Science Laboratory, York, United Kingdom
Date of delivery as substance B:	19.4.2000
Delivered amount:	0.25 g
Order no.:	2670

Leverkusen, 21.6.00

Dr. J. Keppler