STUDY REPORT

Name and address of sponsor:	Bayer AG Landwirtschaftszentrum Monheim Institut fur Okobiologie 51368 Leverkusen Germany
Sponsor's Representative	[redacted]
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	[redacted]
Study dates:	
Initiation	27 April 2000
Experiment start	8 May 2000
Experiment end	11 May 2000
Study Completion	9 June 2000

Study Number:HT0400a

Substance A:
Acute Contact Toxicity to Honey Bees
(*Apis mellifera*).

1. Summary

Tests were carried out to determine the acute contact toxicity of Substance A to adult honey bees (*Apis mellifera* L.). The protocol followed the EPPO guidelines (1992) and are in accordance with the draft EPA Ecological Effects Test Guidelines (OPPTS 850.3020 Honey Bee Acute Contact Toxicity) and OECD guideline 214 Honeybees, Acute Contact Toxicity (September 1998). All doses and toxicity data for the test substance refer to Substance A as the active ingredient.

Three batches of bees, in groups of 10 bees, were topically dosed on the thorax with 1µl drops containing 140, 110, 78, 56, or 40 ng Substance A /bee in acetone. Mortality and sub-lethal effects were assessed at 4, 24 and 48 hours after dosing. Results indicated that the 24-hour contact LD_{50} of Substance A is greater than 140 ng/bee but by 48 hours and 72 hours this had decreased to 50 ng/bee and 49 ng/bee respectively. There were significant sublethal effects in all doses at 4 hrs with recovery or death by 48 hrs.

2. Introduction

This study was carried out on behalf of Bayer AG to establish the acute contact toxicity of Substance A to adult worker honey bees 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 contact exposure are assessed by cuticular absorption following the application of a droplet to the body surface of a solution of the test substance in deionised water.

The protocol followed the European Plant Protection Organisation guidelines (1992) and methods are in accordance with the draft EPA Ecological Effects Test Guidelines (OPPTS 850.3020 Honey Bee Acute Contact Toxicity) and OECD guideline 214 Honeybees, Acute Contact Toxicity (September 1998). All doses and toxicity data for the test substance refer to Substance A as the active ingredient.

3. Materials and methods

3.1 Test substance

Substance A 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 NBU83. The test substance was stored, as recommended by the sponsor, in the dark at approx. 4°C.

Substance A, a white solid, was dispersed in acetone for contact dosing for a maximum of two hours before use.

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) until needed 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 for the test, with three 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 intervals of 4, 24, 48 and 72 hours after dosing.

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 A were checked visually during immediately before use. Solutions of the test doses were mixed well immediately prior to use and were homogenous for the purpose of administration.

The top doses were made by preparing a solution containing 1390 µg/ml Substance A in acetone. Control bees were treated with acetone.

The test were started on 11 May 2000 using the dose levels in Table 1 and the timetable is shown in Appendix 1.

Table 1: Contact test dose levels.

Dose group	Substance A ng /bee
1	140
2	110
3	78
4	56
5	40
Control	Acetone

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 bee was dosed on the thorax with a 1 µl drop of a given pesticide concentration or 1 µl drop of acetone using an Anachem EDP Plus micropipette. The lid was placed on the cage, the bees were allowed to recover and kept in the incubator with a continuous supply of 50% w/v aqueous sucrose solution as food.

3.4 Analysis of data

The mortality results of the tests with Substance A were analysed using the CSL probit programme (Probit 1, version 4).

4. Results

Substance A readily dissolved in acetone at 1390 µg/ ml to give a clear, colourless solution.

The results for the contact test are listed in Table 2 for Substance A. Sub-lethal effects recorded are shown in Table 3. The 48-hour results were different from those at 24 hours, indicating there were delayed effects and the test was extended to 72 hours.

The 24-hour, 48-hour and 72-hour LD_{50} for Substance A are given in Table 4.

With Substance A the 24-hour LD_{50} is greater than 140 ng/bee but large numbers of bees in all but the lowest dose level showed significant sub-lethal effects. The 48-hour and 72-hour LD_{50}'s were 50 ng /bee and 49 ng/ bee Substance A respectively. Control mortality was 3% at 24, 48 and 72 hours.

TABLE 2. Results of contact dosing tests with Substance A.

Dose	Cage	Number dead (n=10)			
(ng/bee)	No.	4 hours	24 hours	48 hours	72 hours
140	69	0	3	7	10
140	70	0	3	9	10
140	71	0	3	7	10
110	72	0	8	9	9
110	73	0	8	10	10
110	74	0	7	10	10
78	75	0	2	3	3
78	76	0	2	10	10
78	77	1	3	8	8
56	78	0	5	6	6
56	79	0	3	9	10
56	80	0	0	3	3
40	81	0	2	4	4
40	82	0	5	5	6
40	83	0	2	2	2
0	84	0	0	0	0
0	85	0	0	0	0
0	86	0	1	1	1

TABLE 3. Sub-lethal effects observed in contact dosing tests with Substance A.

Dose	Cage	Number knockdown (K) or stumbling (S) (n=10)			
(ng/bee)	No.	4 hours	24 hours	48 hours	72 hours
140	69	4K	2S, 5K	1S, 1K	0
140	70	3S, 1K	2S, 5K	1K	0
140	71	2S, 5K	1S, 3K	2K	0
110	72	6K	2K	0	0
110	73	1S, 7K	1S, 1K	0	0
110	74	3S, 7K	1S, 2K	0	0
78	75	5K	2K	0	0
78	76	7K	3S, 5K	0	0
78	77	2S, 7K	3S	1K	0
56	78	5K	5K	0	0
56	79	7K	1S, 1K	0	0
56	80	9K	2S, 5K	0	0
40	81	2K	1S, 2K	0	0
40	82	8K	0	0	0
40	83	2S, 5K	0	0	0
0	84	0	0	0	0
0	85	0	0	0	0
0	86	0	0	0	0

Table 4. Contact toxicity of Substance A

Time	LD_{50} (ng Substance A ai/bee)	95% Confidence Interval for LD_{50}	Estimate of slope of response line
24 hours	>140	NE*	1.1
48 hours	50	9.1-71	2.6
72 hours	49	3.3 - 71	4.4

* could not be estimated from the data

5. Discussion and conclusions

With Substance A the 24-hour LD_{50} is greater than 140 ng/bee but large numbers of bees in all but the lowest dose level showed significant sub-lethal effects. The 48-hour and 72-hour LD_{50}'s were 50 ng /bee and 49 ng/bee Substance A respectively. The 48-hour results were less than those at 24 hours, indicating there were delayed effects.

7. References

1977 Stevenson, J H, Needham, P and Walker, J.
"Poisoning of honey bees by pesticides - Investigation of the changing pattern in Britain over 20 years"
Rothamsted Annual Report 1977 Part II pages 55-72

1992 European and Mediterranean Plant Protection Organisation
"Guideline on test methods for evaluating the side-effects of plant protection products on honey bees"
EPPO Bulletin 22, 203-215

1996 Ministry of Agriculture, Fisheries and Food (UK), Pesticides Safety Directorate and the Health and Safety Executive,
"The Registration Handbook Volumes 1 and 2, Pesticides, Biocides, Plant Protection Products, A guide to the policies, procedures and data requirements relating to their control within the United Kingdom"

September 1998 OECD
"OECD Guidelines for the testing of chemicals. Guideline 214. Honeybees, acute contact toxicity test."

Distribution

Sponsor
Study Director [redacted]

APPENDIX I

ACTUAL TIMETABLE FOR THE STUDY

ACUTE CONTACT HONEYBEE TOXICITY TEST HT0400a

Day	Time (hours)	Date & Actual Time	Activity
-3	pm	8/05/00	Dispense test substance
0	-2½	11/05/00	Start bee collection
	-2	11/05/00	Stock prepared and dilutions started
	-1	11/05/00 09:35	Bees in incubator
	-1	11/05/00 09:18	Dilutions finished
	0	11/05/00 10:23	Contacts dosed
	+4	11/05/00 14:21	Assess contacts
1	+24	12/05/00 10:37	Assess contacts
2	+48	13/05/00 10:33	Assess contacts
3	+72	14/05/00 10:01	Assess contacts

STUDY REPORT AMENDMENT 1

Name and address of sponsor:	Bayer AG Landwirtschaftszentrum Monheim Institut für Ökobiologie 51368 Leverkusen Germany
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(*Apis mellifera*).

Amendment 1
Change from deionised water to acetone as solvent
In the introduction the reference to dispersion of the test substance in deionised water should read acetone. All test substance solutions were in acetone.

Amendment 2
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. HT0400a

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:	Central Science Laboratory, York, United Kingdom
Date of delivery as substance A:	19.4.2000
Delivered amount:	1.15 g
Order no.:	2670

Leverkusen, 21.6.00