Data Evaluation Report on the acute contact and oral toxicity of BAS 670 00 H to the honey bee
PMRA Submission Number 2003-0839 **EPA MRID Number 459018-14**

Data Requirement: PMRA DATA CODE: 9.2.4.1(acute contact); 9.2.4.2 (acute oral)
EPA DP Barcode: D290076
OECD Data Point: 8.7.2; 8.7.1
EPA Guideline: OPPTS 850.3020 (contact); OPP 141-1 (contact)

Test material: BAS 670 00 H **Purity (%):** 351.5 g a.i./L (31% a.i.)
Common name: BAS 670H
Chemical name:
IUPAC: [3-(4,5-dihydro-iosoxazol-3-yl)-4-methane-sulfonyl-2-methyl-phenyl]-(5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone
CAS name: [3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)-
CAS No.: 210631-61-8
Synonyms: Reg. No. 375080, methanone

Primary Reviewer (officer number): 1268
PMRA
Signature:
Date: August 18, 2004

Secondary Reviewer (officer number): 1247
PMRA
Date: August 23, 2004

Secondary Reviewer: Stephen Carey, Biologist
EPA
Signature: Stephen Carey
Date: February 24, 2005

Company Code: BAZ
Active Code: MTN
Use Site Category: 14
EPA PC Code: 123009

CITATION: Schmitzer, S. 2000. Effects of BAS 670 00 H (acute contact and oral LD50) on honey bees (*Apis mellifera* L) (Hymenoptera, Apidae) in the laboratory. Institut für Biologische Analytik and Consulting IBACON, GmbH. IBACON Project no. 7625036; BASF AG, Germany, October 26, 2000.

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EXECUTIVE SUMMARY:
In a 48-hr limit-test, honey bees (*Apis mellifera L.*) were exposed to BAS 670 00 H (guarantee 351.5 g a.i./L [measured], equivalent to 31% a.i.) at a concentration of 100 µg EP/bee (nominal) in a dermal contact test, and 108.3 µg EP/bee (mean measured) in an oral toxicity test. Tests were conducted according to OECD Guidelines 213 and 214. Female bees were approximately 4-6 weeks old at test exposure. In the contact assay, the test substance was dissolved in water and 0.6% Adhäsit (a non-toxic wetting agent), and placed on the ventral thorax of the bee with an applicator. In the oral test, the substance was mixed in food (50% final sugar solution) and offered in syringes for a period of not exceeding 105 minutes. Results with a toxic standard (dimethoate) showed that the tests were adequately sensitive. Mortality and behavioural abnormalities were assessed at 4, 24 and 48 h after dosing.

Acute contact: No mortality or significant behavioural effects were observed after 48 h exposure to 100 µg EP/bee. The NOEL and LD_{50} values were 100 and >100 µg EP/bee, respectively. For PMRA, the test material is classified as relatively non-toxic to honey bees in accordance with the classification of Atkins (1981) and for EPA, the test material is classified as practically non-toxic to honey bees in accordance with the classification system of the U.S. EPA.

The acute contact study is classified as acceptable and provides supplementary data for the PMRA guideline requirement for an acute contact toxicity study for honey bees (DACO 9.2.4.1). The study is scientifically sound and fulfills the USEPA guideline requirement for a honey bee contact toxicity test (§141-1). This study is classified by the USEPA as acceptable for a formulated product.

Acute oral: No mortality or significant behavioural effects were observed after 48 h exposure to 108.3 µg EP/bee. The NOEC and LC_{50} values were 108.3 and >108.3 µg EP/bee, respectively. The acute oral study is scientifically sound, but does not fulfill any current U.S. EPA guideline requirement. It is classified as acceptable and provides supplementary data for the Canadian guideline requirement for an acute oral toxicity study for honey bees (DACO 9.2.4.2). The acute oral study provides useful information on the acute oral toxicity of the end-use product BAS 670 00 H (31% a.i.) to the honey bee.

Results Synopsis

Test organisms:	Honey bee (*Apis mellifera*), adult worker female bees
Test organism age:	4 - 6 weeks old.
Test Type:	Acute contact and oral toxicity limit test.

Acute contact

48 hr LD_{50}: >100 µg EP/bee 95% C.I.: n/a
48 hr NOEL: 100 µg EP/bee
Probit Slope: n/a 95% C.I.: n/a
Endpoint(s) Effected: none

Acute oral

48 hr LC_{50}: >108.3 µg EP/bee 95% C.I.: n/a
48 hr NOEC: 108.3 µg EP/bee
Probit Slope: n/a 95% C.I.: n/a
Endpoint(s) Effected: none

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: OECD Guidelines 213 and 214, and recent recommendations of the ICPBR group, held in Avignon, France, 1999. No deviations were identified.

COMPLIANCE: Study conducted according to OECD (1997) and Chemikaliengesetz (Chemicals Act, Annex 1) (1994/97) GLP standards. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material BAS 670 00 H

Description: grey-beige liquid

Lot No./Batch No. : 2000-1

Purity: 351.5 g a.i./L (analytical; 31% a.i.)

Stability of Compound Under Test Conditions: considered stable in water; expiry March 2002

The stability of BAS 670 00 H in sugar water for the 105-minute open access feeding period was not reported.

Storage conditions of test chemicals: in original container at 5 - 30°C

Density: 1.134 g/mL

Physicochemical properties of BAS 670 H (active ingredient of BAS 670 00 H).

Parameter	Values	Comments
Water solubility at 20°C	510 mg/L in deionized H_2O at 20°C >100 g/L at pH >9	Highly soluble
Vapour pressure	$<1.0 \times 10^{-12}$ mbar (= $<1.01 \times 10^{-10}$ Pa) at 20°C	Low volatility
UV absorption	207 nm: 0.7637 272 nm: 0.2426 300 nm: 0.1636 410 nm: 0.0027	Potential for phototransformation (i.e. absorbance occurring within 285 - 350 nm range)
pKa	4.06 @ 20°C	Dissociated at environmentally relevant pHs
Log Kow	-1.52 @ 20°C	Not likely to bioaccumulate

2. Test organism:

Species: Honey bee (*Apis mellifera*)
Age at test initiation: Female worker bees 4 - 6 weeks old
Source: Honey bee colonies bred by testing facilities
Date of collection: Not provided

Cultural Background: Colonies disease-free and queen-right

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: None - main study is a limit test.

b) Definitive Study

Table 1. Experimental Parameters/Design

Parameter	Value	Remarks *Criteria*
Acclimation: Duration: Feeding: Health of bees	none indicated	O.K. *EPA : No acclimation period is necessary* *Health of bees: Disease free*
Cage - description and size	stainless steel, perforated w/ ventilation holes 10 cm x 8.5 cm x 5.5 cm (l x w x h)	O.K. *EPA : Test chambers may be constructed of metal, plastic, wire mesh, or cardboard. A vial containing sugar water must be attached.*
Test conditions		O.K.
Temperature: Humidity: Lighting:	26 - 27°C 50 - 85% darkness (except during observation periods)	

Parameter	Value	Remarks *Criteria*
		Temperature: EPA requires 25-35°C *OECD requires 25±2°C* *Humidity* *EPA requires 50 - 80% humidity* *OECD requires 50 - 70% humidity* *Lighting* *EPA/OECD recommend darkness except during dosing and observation.*
Solvent/dispersant control, if used Name: Concentration:	water + 0.6% Adhäsit	O.K. *EPA/OECD prefer acetone as a solvent* *EPA: negative and solvent controls required. Positive control not required.*
Number of bees per cage	10	O.K. *EPA requires at least 25 bees per treatment* *OECD prefers 10 bees per cage*
Number of cages per treatment	5	O.K. *EPA: One cage per each treatment level and each control.*
Number of replicates Negative control: Solvent/dispersant control, if used: Treated:	 5 5 5	O.K. *OECD requires at least three replicate, each of ten bees* *EPA: Replications are not required.*

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Parameter		Value	Remarks *Criteria*
For **Acute contact** study	Doses used Nominal: Measured:	100 µg EP/bee	O.K. for limit test *EPA requires at least five dosage levels, spaced geometrically at least 60% of the next higher level* *OECD requires five doses in a geometric series, with a factor not exceeding 2.2*
	Method of test material application including the body part and volume of test solution applied	a single 5 µL droplet of BAS 670 00 H in solvent placed on the ventral thorax using a Burkhard Applicator	O.K. *EPA: Test material administered as single topical dose (topical drop) or whole body exposure to impregnated dust.*
	Time of test material application	not provided	
For **Acute oral** study	Doses used Nominal: Measured:	100 µg EP/bee 108.3 µg EP/bee	O.K.
	Details of the food source	commercial syrup (30% Saccharose, 31% glucose, 39% fructose)	
Method of feeding during the study		given directly after treatments in syringes *ad libitum*	*EPA: A 50% sugar/water solution will be provided ad libitum throughout the holding and test periods. A vial containing solution must be attached to each cage.*

Parameter	Value	Remarks *Criteria*
Duration of the study	48 h	O.K. *EPA: 48 hours with observation for mortality and signs of intoxication at 4, 24, and 48 hours after exposure to test material.*
Indicate other factors, if any		
Reference chemical, if used Name: Concentration(s):	 dimethoate (395.7 g/L analytical; 400 g/L nominal) 0.05, 0.1, 0.2 and 0.4 µg a.i./bee (contact test) 0.04, 0.08, 0.16, 0.33 µg a.i./bee (oral test)	O.K.

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including sublethal effects/toxicity symptoms	mortality, behavioural abnormalities	O.K. 0% mortality in controls from both studies. *EPA requires less than 20% mortality in the controls* *OECD requires less than 10% mortality in the controls*
Observation intervals	4, 24 and 48 h	O.K. *EPA /OECD require observation intervals of 4, 24 and 48 h after dosing*
Amount of treated diet consumed per group (For acute oral)	21 - 22 mg solution per bee	

Parameters	Details	Remarks *Criteria*
Were raw data included?	yes	
Other observations, if any		

II. RESULTS AND DISCUSSION:

A. MORTALITY: No mortality was observed in bees exposed to 108.3 µg EP/bee in the oral test. In the contact test one bee died after 24 hours in the 100 µg EP/bee treatment. No mortality was observed in negative controls from either study.

Results with dimethoate toxic standards:

Acute Contact Test
24-h LD_{50}: 0.21 ug a.i./bee 95% CI: 0.18 - 0.24 ug a.i./bee
48-h LD_{50}: 0.19 ug a.i./bee 95% CI: 0.17 - 0.22 ug a.i./bee
Accepted range: 0.10 - 0.30 ug a.i./bee

Acute Oral Test
24-h LC_{50}: 0.14 ug a.i./bee 95% CI: 0.12 - 0.15 ug a.i./bee
48-h LC_{50}: 0.13 ug a.i./bee 95% CI: 0.12 - 0.14 ug a.i./bee
Accepted range: 0.10 - 0.35 ug a.i./bee

B. SUB-LETHAL TOXICITY EFFECTS:

No behavioural effects were observed in control chambers in either the contact or oral test. In the contact test, one exposed bee exhibited apathy after 24 h, and in the oral test, one exposed bee exhibited coordination problems and apathy after 48 h. In the oral test, there were no apparent palatability issues as food consumption was similar between bees exposed to control and test material (21 - 22 mg/bee in test material, vs. 22 - 23 mg/bee in controls).

C. REPORTED STATISTICS: An $L(E)C_{50}$ was not be determined as less than 50% mortality or sublethal effects occurred in either of the contact or oral limit tests. The NOEC was visually determined to be equal to the test concentrations. The contact and oral LD50 (24 and 48 h) of the toxic standard were estimated by Probit Analysis using EASY ASSAY© Critical Values, v.3.01.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER: The reviewer has no objections to the statistics used by the study authors.

E. STUDY DEFICIENCIES: None. - All test validity requirements were met.

F. REVIEWER'S COMMENTS: None.

G. CONCLUSIONS: This study is scientifically sound for both the acute contact and oral toxicity tests. It fulfills the data requirements for an acute contact toxicity test with the honeybee (PMRA DACO 9.2.4.1 and 9.2.4.2 and EPA §141-1) and an acute oral toxicity test (PMRA DACO 9.2.4.2, no comparable EPA guideline). The study is classified as acceptable for the selected guideline requirements which it fulfills. The acute contact LD_{50} and NOEL were >100 µg EP/bee and 100 µg EP/bee, respectively. The acute oral LC_{50} and NOEC were >108.3 µg EP/bee and 108.3 µg EP/bee, respectively.

For acute contact

LD_{50}: >100 µg EP/bee
Slope: n/a
NOEL: 100 µg EP/bee

For acute oral

LC_{50}: >108.3 µg EP/bee
Slope: n/a
NOEC: 108.3 µg EP/bee

III. REFERENCES:

Atkins, E. L., Kellum, D. and Atkins, K. W. 1981. Reducing pesticide hazards to honey bees: Mortality prediction techniques and integrated management strategies. University of California, Division of Agriculture Science, Leaflet 2883. P 22.

Approved 04/01/01 C.K.

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