

MRID No.: 436781-67

DATA EVALUATION RECORD
§ 141-1 - HONEY BEE ACUTE CONTACT AND ORAL LD₅₀ TEST

1. **CHEMICAL:** Azoxystrobin PC Code No.: 128810

2. **TEST MATERIAL:** ICIA5504 WG formulation Purity: 51.6%

3. **CITATION**

Authors: Gough, H.J. and D. Jackson
Title: ICIA5504: Acute Contact and Oral Toxicity to Honey Bees (*Apis mellifera*) of a 500 g kg⁻¹ WG Formulation

Study Completion Date: October 26, 1994

Laboratory: Jealott's Hill Research Station,
 Bracknell, Berkshire, UK

Laboratory Report ID: RJ1720B

Sponsor: Zeneca Inc., Wilmington, DE

MRID No.: 436781-67

4. **REVIEWED BY:**

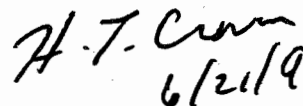
William Erickson
 Biologist
 EEB/EFED/EPA

Signature: 

Date: 4/01/96

5. **APPROVED BY:**

Harry Craven
 Section Head 4
 EEB/EFED/EPA

Signature: 

Date: 6/21/96

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Apis mellifera*
Definitive Study Duration: 48 hours

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirement. Based on nominal concentrations, the acute contact and oral LD₅₀ was greater than 200 µg ai/bee, which classifies ICIA5504 WG formulation as practically nontoxic to the honey bee.

①

Results Synopsis

LD₅₀: >200 µg ai/bee

95% C.I.: N/A

NOEL: 200 µg ai/bee

Probit Slope: N/A

8. ADEQUACY OF THE STUDY

A. Classification: Core for a formulated product.

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. Age of the test bees was not reported.
2. The number of bees (10) per replicate (cage) was less than recommended (25 bees per replicate).

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species: Honey bee (<i>Apis mellifera</i>).	<i>Apis mellifera</i>
Age at beginning of test: Worker bees of uniform age.	Not reported
Supplier	Jealott's Hill Research Station, Bracknell, Berkshire, UK
All bees from the same source?	Yes, bees from hive 1991/12.

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	Yes

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~~129081~~
2. **TEST MATERIAL:** ICIA5504 WG formulation **Purity:** 51.6%

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BP Barcode: ~~D217072, D217078~~
MRID No.: 436781-67

4. **REVIEWED BY:** Max A. Feken, M.S., Environmental Toxicologist, KBN Engineering and Applied Sciences, Inc.

Signature:

Date: 1/23/96

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.

Signature:

Date: 1/23/96

5. **APPROVED BY:**

Signature:

Date:

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Apis mellifera*
Definitive Study Duration: 48 hours

7. **CONCLUSIONS:** This study is scientifically sound and meets guideline requirements. Based on nominal concentrations, the acute contact and oral LD₅₀ was greater than 200 µg ai/bee, which classifies ICIA5504 WG formulation as practically non-toxic to the honey bee. The NOEL was determined to be 200 µg ai/bee.

Guideline Criteria	Reported Information
Lighting: Bees should be maintained in the dark	Bees maintained in the dark except during observations.
Temperature: 27°C (80°F).	24-25°C
Relative humidity: Approx. 65%	49-62% relative humidity

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	No
Reference toxicant tested?	Yes, dimethoate.
Method of administration: Whole body exposure in a nontoxic dust diluent; or topical exposure via microapplicator.	Contact test: Topical exposure via micro-syringe (Burkard micro-applicator). Oral test: Dose administered via feeding tube.
Definitive Test Nominal doses: Sufficient number of dosage levels to yield statistically sound data unless it can be determined that the LD ₅₀ will be greater than 25 µg/bee.	Nominal concentrations of 5, 10, 20, 50, 100, and 200 µg ai/bee for both tests.
Controls: Negative control and/or diluent/solvent control	Diluent control for the contact test and negative control for the oral test.
Number of bees per cage: 25 (recommended)	10 bees per cage (both tests).
Number of cages per group: 3 replicate cages per group is recommended.	3 cages per treatment group (both tests).

Guideline Criteria	Reported Information
Carrier: Non-toxic dust (e.g, Pyrolite).	Contact test: Deionized water with 500 mg/L 'Agral' as a surfactant to aid spreading of the drop. Oral test: N/A.
Solvent: Distilled water or the following solvents: dimethyl-formamide, triethylene glycol, methanol, acetone, ethanol.	N/A.
Volume of test solution: ≤2 µl/bee (for contact tests).	Contact test: 1 µl drop. Oral test: 0.2 ml (total volume) per cage or 0.02 ml per bee. Test solution contained the test formulation dissolved in 50% sucrose solution.
Observations period: At least 48 hours.	48 hours for both tests.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Controls: Mortality not more than 15%	0% for acute contact and 13% for oral toxicity test.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality - Contact Test

Dosage ($\mu\text{g ai/bee}$)	No. of Bees	Rep	Cumulative Number of Dead				
			Hour of Study				
			1	2	4	24	48
Diluent Control	10	a	0	0	0	0	0
		b	0	0	0	0	0
		c	0	0	0	0	0
5	10	a	0	0	0	0	1
		b	0	0	1	1	1
		c	0	0	0	0	0
10	10	a	0	0	0	0	0
		b	0	0	0	0	1
		c	0	0	0	0	0
20	10	a	0	0	2	6	6
		b	0	0	0	1	1
		c	0	0	0	0	2
50	10	a	0	0	0	1	1
		b	0	0	0	0	0
		c	0	0	0	0	0
100	10	a	0	0	0	0	0
		b	0	0	0	0	0
		c	0	0	0	0	2
200	10	a	0	0	0	0	1
		b	0	0	0	0	0
		c	0	0	0	0	0

Other Significant Results: Although at least one mortality was observed at each dosage level, the mortalities were not indicative of a positive dose response. Only one mortality was noted at the highest treatment level (200 $\mu\text{g ai/bee}$). There were only a few sublethal effects noted at various dosage levels including the control. During the same month using the same colony of bees, an LD_{50} was obtained using dimethoate as a toxic standard in order to determine if the bees were reacting normally to pesticide doses under similar conditions. Dimethoate produced a positive dose-response and an LD_{50} value comparable to the mean LD_{50} calculated using data collected from 1981 to 1992.

6

Mortality - Oral Test

Dosage ($\mu\text{g ai/bee}$)	No. of Bees	Rep	Cumulative Number of Dead				
			Hour of Study				
			1	2	4	24	48
Control	10	a	0	1	1	1	1
		b	1	1	1	1	2
		c	0	0	0	1	1
5	10	a	0	0	0	0	0
		b	0	0	0	1	1
		c	0	1	1	2	4
10	10	a	0	0	0	2	4
		b	2	2	2	2	2
		c	0	0	0	0	0
20	10	a	0	1	1	1	2
		b	1	1	1	1	2
		c	0	0	0	0	1
50	10	a	0	1	1	1	3
		b	0	0	0	0	0
		c	0	0	0	0	1
100	10	a	0	0	0	0	1
		b	0	0	0	0	2
		c	0	0	0	0	1
200	10	a	0	0	0	0	1
		b	0	0	0	2	4
		c	0	0	0	0	1

Other Significant Results: No positive dose-related response was evident in this test.

Reported Statistical Results - Contact Test

Statistical Method: By visual inspection

LD₅₀: >200 $\mu\text{g ai/bee}$

95% C.I.: N/A

NOEL: 200 $\mu\text{g ai/bee}$

Probit Slope: N/A

Reported Statistical Results - Oral Test

Statistical Method: By visual inspection

LD₅₀: >200 $\mu\text{g ai/bee}$

95% C.I.: N/A

NOEL: 200 $\mu\text{g ai/bee}$

Probit Slope: N/A

13. Verification of Statistical Results - Contact Test

Statistical Method: Williams' test

LD₅₀: >200 µg ai/bee

95% C.I.: N/A

NOEL: 200 µg ai/bee

Probit Slope: N/A

Verification of Statistical Results - Oral Test

Statistical Method: Williams' test

LD₅₀: >200 µg ai/bee

95% C.I.: N/A

NOEL: 200 µg ai/bee

Probit Slope: N/A

14. REVIEWER'S COMMENTS: This study is scientifically sound, meets the guideline requirements for honey bee acute contact and oral toxicity tests, and can be classified as Core for a formulated product. Based on nominal concentrations, the acute contact and oral LD₅₀ was greater than 200 µg ai/bee, which classifies ICIA5504 WG formulation as practically non-toxic to *Apis mellifera*. The NOEL was determined to be 200 µg ai/bee.

ICIA5504 FORMULATION
 File: C:43678167.

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	CONTROL	3	0.000	0.159	0.159
2	5	3	0.067	0.267	0.240
3	10	3	0.033	0.213	0.240
4	20	3	0.300	0.557	0.311
5	50	3	0.033	0.213	0.311
6	100	3	0.067	0.260	0.311
7	200	3	0.033	0.213	0.311

ICIA5504 FORMULATION
 File: C:43678167.

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
CONTROL	0.159				
5	0.240	0.676		1.76	k= 1, v=14
10	0.240	0.676		1.85	k= 2, v=14
20	0.311	1.262		1.88	k= 3, v=14
50	0.311	1.262		1.89	k= 4, v=14
100	0.311	1.262		1.90	k= 5, v=14
200	0.311	1.262		1.91	k= 6, v=14

s = 0.148

Note: df used for table values are approximate when v > .20.

ICIA5504 FORMULATION - ORAL TEST

File: 43678167

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	CONTROL	3	0.867	0.867	0.867
2	5	3	0.833	0.833	0.840
3	10	3	0.800	0.800	0.840
4	20	3	0.833	0.833	0.840
5	50	3	0.867	0.867	0.840
6	100	3	0.867	0.867	0.840
7	200	3	0.800	0.800	0.800

ICIA5504 FORMULATION - ORAL TEST

File: 43678167

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
CONTROL	0.867				
5	0.840	0.226		1.76	k= 1, v=14
10	0.840	0.226		1.85	k= 2, v=14
20	0.840	0.226		1.88	k= 3, v=14
50	0.840	0.226		1.89	k= 4, v=14
100	0.840	0.226		1.90	k= 5, v=14
200	0.800	0.564		1.91	k= 6, v=14

s = 0.145

Note: df used for table values are approximate when v > 20.

10

ICIA5504 FORMULATION - ORAL TEST

File: 43678167

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	CONTROL	3	0.867	1.202	1.202
2	5	3	0.833	1.182	1.182
3	10	3	0.800	1.135	1.177
4	20	3	0.833	1.154	1.177
5	50	3	0.867	1.217	1.177
6	100	3	0.867	1.202	1.177
7	200	3	0.800	1.128	1.128

ICIA5504 FORMULATION - ORAL TEST

File: 43678167

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
CONTROL	1.202				
5	1.182	0.125		1.76	k= 1, v=14
10	1.177	0.159		1.85	k= 2, v=14
20	1.177	0.159		1.88	k= 3, v=14
50	1.177	0.159		1.89	k= 4, v=14
100	1.177	0.159		1.90	k= 5, v=14
200	1.128	0.476		1.91	k= 6, v=14

s = 0.189

Note: df used for table values are approximate when v > 20.

11