# Data Evaluation Report on the contact and oral toxicity of pyroxsulam (XDE-742) to the honey bee

**PMRA Submission Number 2006-4727; ID 1283188** **EPA MRID Number ~~469084-xx~~ 469085-08 APVMA ATS 40362**

| | | |
|---|---|---|
| **Data Requirement:** | PMRA DATA CODE: | 9.2.4.1(acute contact); 9.2.4.2 (acute oral) |
| | EPA DP Barcode: | D332116 |
| | OECD Data Point: | IIA 8.7.1 (acute oral) and IIA 8.7.2 (acute contact) |
| | EPA Guideline: | {non-guideline (oral); 141-1 or 850.3020 (contact)} |

**Test material:** Pyroxsulam or XDE-742/BAS770H **Purity (%):** 98 % w/w

Common name: XR-742, X666742 (DowAgroSciences Test Substance Distribution Certificate)

Chemical name: 3-pyridinesulfonamide, N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)

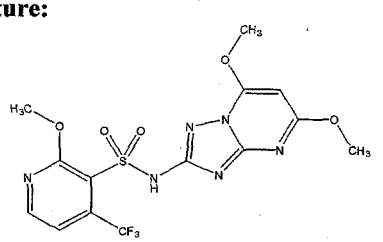
IUPAC: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide

CAS name: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide

CAS No.: 422556-08-9

Synonyms: XR-742, X666742

**Chemical Structure:**



**Primary Reviewer:** Daryl Murphy D. Murphy 22/02/08 **Date:** 29 March, 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

**Secondary Reviewers:** Jack Holland 22/2/08 **Date:** 29 March, 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

Ann Lee (#1369) Emilie Lanière for Ann Lee 05/03/08 **Date:** 08 May, 2007
Environmental Assessment Directorate, PMRA

Christopher Salice **Date:** 20 June 2007
Environmental Fate and Effects Division, U.S. Environmental Protection Agency 04/14/08

**Company Code:** DWE
**Active Code:** JUA
**Use Site Category:** 13, 14
**EPA PC Code:** 108702

**CITATION:** Schmitzer, S. 2004. Effects of XDE-742/BAS77OH (*Acute contact and oral*) on honey bees (*Apis mellifera L.*) in the laboratory. Institut für Biologische Analytik Und Consulting IBACON GmbH, Arheilger Weg 17, 64380, Rossdorf, Germany. Project Number 18361035. Dow AgroSciences European Development Centre, 3 Milton Park, Abingdon, Oxon OX14 4RN, United Kingdom. January 20 2004. Unpublished report.

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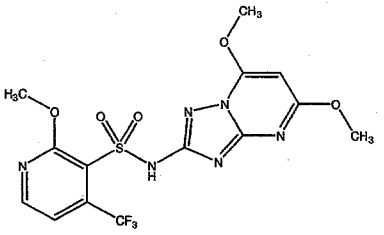


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**EXECUTIVE SUMMARY:**

In a 48 h acute oral and contact toxicity study, honey bees (*Apis mellifera* L.) were exposed to pyroxsulam (98%) administered either in a commercial sugar syrup/water solution at an application rate of 0 (negative control), 0 (5% acetone solvent control) and 107.4 µg pyroxsulam/bee in accordance with OECD 213 Honeybees, Acute Oral Toxicity Test or, topically to the ventral thorax region of anaesthetised bees at an application rate of 0 (negative control), 0 (acetone solvent control) and 100 µg pyroxsulam/bee in accordance with OECD 214 Honeybees, Acute Contact Toxicity Test. In the oral toxicity study, no mortality or sublethal effects were observed in any of the control or treatment groups over 48 hours. The 48 hour oral NOEC (reviewer established) and $LC_{50}$ were 107.4 and >107.4 µg pyroxsulam/bee, respectively. For the contact study, percentage mortalities in the negative control, solvent control and 100 µg pyroxsulam/bee treatment groups were 2, 0 and 0, respectively, after 48 hours. No sublethal effects were observed. The 48 hour contact NOEL (reviewer established) and $LD_{50}$ were 100 and >100 µg pyroxsulam/bee, respectively. Honey bees exposed to the toxic reference chemical, dimethoate, via the contact or oral route exhibited up to 98% mortality over 48 hours exposure. Sublethal effects seen in these dimethoate exposed honey bees were movement coordination problems and apathy.

The test material is classified as very slightly toxic to honey bees in accordance with the classification system used by the Australian Government Department of the Environment and Water Resources.

This study is classified as acceptable and satisfies the guideline requirement for an acute contact and acute oral toxicity study for honey bees. The EPA secondary reviewer stated that the 48 h acute oral toxicity study in honey bees (*Apis mellifera* L.) is classified as Supplemental since EPA does not require oral toxicity studies in honey bees and that the study may be useful for risk assessment purposes.

**Results Synopsis**

| | |
|---|---|
| Test organisms and test organism age: | Honey bee (*Apis mellifera* L.), 4-6 weeks old female adult bees |
| Test Type: | Acute Contact and Acute Oral Toxicity Tests |
| **For acute contact toxicity** | |
| 48 hr $LD_{50}$: | >100 µg pyroxsulam/bee |
| | 95% C.I. Not applicable |
| 48 hr NOEL: | Not reported by the study, set at 100 µg pyroxsulam/bee by the study reviewer. |
| | Probit Slope and 95% C.I: Not applicable |
| Endpoint(s) Effected: | Mortality and sublethal effects. |
| **For acute oral toxicity** | |
| 48 hr $LD_{50}$: | >107.4 µg pyroxsulam/bee |
| | 95% C.I. Not applicable |
| 48 hr NOEL: | Not reported by the study, set at 100 µg pyroxsulam/bee by the study reviewer. |
| | Probit Slope and 95% C.I.: Not applicable |
| | 95% C.I.: Not applicable |
| Endpoint(s) affected: | Mortality. |

## I. MATERIALS AND METHODS

### GUIDELINE FOLLOWED:

This study was designed to comply with the following internationally accepted guidelines and recommendations:

- OECD 213: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Oral Toxicity Test, (adopted 21st September 1998)
- OECD 214: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Contact Toxicity Test, (adopted 21st September 1998)
- Recommendations of the ICPBR group, held in Avignon, France, 1999

A number of deviations from the Guidelines were identified relating mainly to procedural issues. These deviations are to be found in Table 13 on page 27 of this draft DER.

### COMPLIANCE:

The study was performed in compliance with:

- The OECD Principles of Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17
- Chemikaliengesetz ('Chemicals Act') der Bundesrepublik. Deutschland (ChemG), Anhang 1 ('Annex 1'), 2002
- Commission Directive 1999/11 EC of 08 March 1999 (Official Journal N° L 77/8)

which are consistent with:

- United States Environment Protection Agency, FIFRA, Title 40 CFR Part 160, Federal Register, 29 November 1983 and subsequent Amendment Federal Register 17 August 1989
- Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984

The study was reported in compliance with the study protocol and the IBACON Standard Operating Procedures. The study and/or test facility were reported as periodically inspected by the Quality Assurance Unit (QAU) and the dates and the phases of the inspections were included into the final report. The data contained with the final report were audited in comparison to the raw data.

A signed and dated GLP compliance statement was included in the final Report.

A signed and dated Quality Assurance Unit Statement was included in the final Report.
(The statement noted that the experimental phase of the study was not inspected, but the processes of the laboratory and of the study involved were inspected in regular intervals).

A signed and dated Statement of No Data Confidentiality Claims was included in the final Report.

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**A. MATERIALS:**

**1. Test Material** XDE-742/BAS770H (referred to as pyroxsulam in this draft DER)

**Description:** A light tan coloured solid

**Lot No./Batch No. :** E0952-52-01

**Purity:** 98% pyroxsulam (based on the certificate of analysis)

**Stability of Compound under Test Conditions:** Not stated.
The study report indicated that stability in acetone was "not indicated" and made reference to seeing the expiry date for formulated product (see Table 13. Deviations from guidelines and other study deficiencies, page 27 of this draft DER).

With respect to the toxic standard, dimethoate, the study report stated it was considered stable under test conditions in water.

**Storage conditions of test chemicals:** Stored in the original container, at ambient conditions (room temperature), in the dark.

**Physicochemical properties of pyroxsulam.**

| Parameter | Values | Comments |
|---|---|---|
| **Water solubility at 20°C** | | |
| **pH 4** | 0.0164 g/L | Turner, 2004 (a) |
| **pH 6** | 0.0626 g/L | Turner, 2004 (a) |
| **pH 7** | 3.2 g/L | Turner, 2004 (a) |
| **pH 9** | 13.7 g/L | Turner, 2004 (a) |
| **Vapour pressure** | <1E-7 Pa | Madsen, 2003 |
| **UV absorption** | Not applicable | |
| **pKa** | 4.670 | Cathie, 2004 |
| **Kow** | | |
| **pH 4** | 12.1 (log Pow = 1.08) | Turner, 2004 (b) |
| **pH 7** | 0.097 (log Pow = -1.01) | Turner, 2004 (b) |
| **pH 9** | 0.024 (log Pow = -1.60) | Turner, 2004 (b) |

**Note: physicochemical data taken from the Study Profile Templates for the acute contact and oral toxicity of pyroxsulam to honey bees (Mercer, 2006a, b) with the information on the UV properties of pyroxsulam reported as not available at the time of publication of the Study Profile Template. Note that the Kow values shown in the study profile template were misordered. The correct values (confirmed by examination of Turner (2004b) in Madsen (2006)) are shown above in the physicochemical properties of pyroxsulam table.**

The study report noted that pyroxsulam was soluble in acetone according to non-GLP pre-experiments for determination of solubility (in that solvent).

**Toxic standard**

| | |
|---|---|
| **Name:** | **Perfekthion EC (BAS 152 11 I)** |
| **Description:** | Blue liquid |
| **Lot No./Batch No. :** | 2002-1 |
| **Active constituent/content:** | 400 g dimethoate/L (nominal) and 401.2 g dimethoate/L (analysed) |
| **Stability of Compound under Test Conditions:** | In water, the toxic standard was considered stable under the test conditions. |
| **Storage conditions of test chemicals:** | Stored in the original container, at ambient conditions (room temperature), in the dark. |

**Adhäsit adhesion aid**

In the contact toxicity test, the tap water used to dissolve the pyroxsulam (with the aid of acetone) contained 1% Adhäsit to improve the spreading of the test droplet on the water repellent hairs on the bees' thoraxes.

| | |
|---|---|
| **Name:** | Adhäsit |
| **Batch No.:** | 0100208 |
| **Active Ingredient/Content:** | 100 g/L Triethanolamin-Dodecylbenzolsulfonat (nominal) |
| **Type:** | Adhesive |
| **Manufacturer:** | Spiess-Urania Chemicals GmbH, Heidenkampsweg 77, 20097 Hamburg |
| **Expiry Date:** | 01/2005 |
| **Storage:** | -10 - +30°C, in the dark |
| **Target Amount in this Study:** | 1% |

**2. Test organism:**

| | |
|---|---|
| **Species** (common and scientific names): | Worker (female) honey bees (Insects, Hymenoptera)<br>*Apis mellifera* L. |
| **Age at test initiation:** | 4-6 weeks old female adult bees<br>The ages of the honeybees tested satisfy OECD 213 and 214's requirement that young, adult worker bees are used for the test. The US EPA OPPTS 850-3020 requirement that the worker bees used for testing should be 1 to 7 days old at test initiation has not been met. As the tests were based on OECD 213 and 214, the apparent failure to use honeybees of 1 to 7 days of age as required by US EPA OPPTS 850-3020 is not considered a deficiency. |
| **Source:** | Honey bee colonies bred by IBACON. |
| **Date of collection:** | Bees were collected on the morning of use from the flight board without anaesthetics with the aid of glass tubes. |
| **Cultural Background:** | Bees were identified as bred by IBACON and as disease-free and queen-right.<br><br>It was not indicated that the bees were kept to standard practices as required by the template but as the bees were bred by IBACON it is expected that standard practices were followed. However, OECD 213 and 214 state that all relevant information on colonies used for collection of test bees, including health, any adult disease, any pre-treatment, etc. must be included in the test report. Consequently, the lack of this information is a deviation from the guidelines (see Table 13, page 27 of this draft DER). |

**B. STUDY DESIGN:**

**1. Experimental Conditions**

**a) Range-finding Study:**

Preliminary range finder tests (non-GLP) were performed in order to select appropriate doses for the main contact and oral toxicity tests. According to the results of these range finder tests, limit tests with 100 µg pyroxsulam/bee were conducted for both the contact and oral definitive toxicity tests. The range finding tests were conducted under the same test conditions as in the main test.

Study details and results for the range finding stud test were reported as:

| **Contact Toxicity Test** | |
|---|---|
| Start of Range Finding Test: | 11 August 2003 |
| End of Range Finding Test: | 13 August 2003 |
| Test Duration: | 48 hours |
| Replicates: | 2 replicates with 10 bees per dose |
| Doses [nominal]: | 100, 50, 10 and 5 µg pyroxsulam/L |

The results reported by the study report for this range finding test are shown in Table 1.

**Table 1. Range finding study results [contact toxicity mortality in %].**

| Dose (µg pyroxsulam/L) | % Mortality at: | | |
|---|---|---|---|
| | 4 hours | 24 hours | 48 hours |
| **Carbon dioxide/Water control** | 0% | 5% | 5% |
| **5** | 0% | 0% | 0% |
| **10** | 0% | 0% | 0% |
| **50** | 0% | 0% | 0% |
| **100** | 0% | 0% | 0% |

**Oral Toxicity Test**

| | |
|---|---|
| Start of Range Finding Test: | 11 August 2003 |
| End of Range Finding Test: | 13 August 2003 |
| Test Duration: | 48 hours |
| Replicates: | 2 replicates with 10 bees per dose |
| Doses [nominal]: | 100, 50, 10 and 5 µg pyroxsulam/L |

The results reported by the study report for his range finding test are shown in Table 2.

**Table 2. Range finding study results [oral toxicity mortality in %].**

| Dose (µg pyroxsulam/L) | % Mortality at: | | |
|---|---|---|---|
| | 4 hours | 24 hours | 48 hours |
| **Water control** | 0% | 0% | 0% |
| **5** | 0% | 0% | 0% |
| **10** | 0% | 0% | 5% |
| **50** | 0% | 0% | 0% |
| **100** | 0% | 0% | 0% |

According to the results of the range finder test, a limit test with 100 µg pyroxsulam/bee was conducted for both the definitive contact and oral toxicity tests.

**b) Definitive Study**

Note that in **Table 3** and **Table 4** (and elsewhere where relevant), the template has references to EPA/OECD requirements. The PMRA has provided advice for other ecotoxicity DERs that these template requirements are outdated and reference is now made to current guidelines. As a result, while the template requirements with respect to the EPA/OECD requirements are still shown in the tables, compliance of the study is judged against the current relevant US EPA, OECD etc. requirements.

**Table 3. Experimental Parameters/Design**

| **Parameter** | **Value** | **Remarks** *Criteria* |
|---|---|---|
| Acclimation:<br>Duration:<br>Feeding:<br><br>Health of bees | <br>No acclimatization was reported.<br>Not applicable, Bees were collected on the morning of use.<br><br>Bees were identified as disease-free and from a queen-right hive. | Requirement considered met.<br>*EPA : No acclimation period is necessary*<br>OECD 213 and 214 refer to collection on the morning of use.<br>*Health of bees: Disease free*<br>OECD 213 and 214 refer to use of bees from adequately fed, healthy, as far as possible disease-free and queen-right colonies (i.e. the hive has an egg laying queen). |
| Cage - description and size | Type: Stainless steel cages<br>Size: 10 cm x 8.5 cm x 5.5 cm<br><br>Front Side: Removable glass sheet<br>Bottom: Perforated with 98 ventilation holes each of diameter 1 mm<br>Inner Walls: Lined with filter paper (Co. Schleicher & Schuell, D-37582 Dassel). | Requirements considered met.<br><br>*EPA: Test chambers may be constructed of metal, plastic, wire mesh, or cardboard. A vial containing sugar water must be attached.*<br><br>OECD 213 and 214 states that any appropriate material can be used, e.g. stainless steel, wire mesh, plastic, disposable wooden cages, etc. with the size of test cages being appropriate to the number of bees. |
| Test conditions<br>Temperature:<br><br><br><br>Humidity: | <br>25°C (Incubator temperature). Temperature measured for the contact and oral tests at 0, 24 and 48 hours. At all times the recorded temperature was 25°C.<br>56-60% in the incubator.<br>Recorded humidity results were: | Requirements considered met.<br>*Temperature:*<br>*EPA requires 25-35□C*<br>*OECD requires 25±2□C*<br><br>*Humidity*<br>*EPA requires 50 - 80% humidity*<br>*OECD requires 50 - 70% humidity.* |

| Parameter | Value | Remarks<br>*Criteria* |
|---|---|---|
| | **Relative humidity (%)**<br>**Time** / **Contact test** / **Oral test**<br>**0** 59 59<br>**24 hours** 60 56<br>**48 hours** 56 60 | |
| Lighting: | The caged bees were kept in darkness (except during observation). | *Lighting*<br>*EPA/OECD recommend darkness except during dosing and observation.* |
| Solvent/dispersant control, if used<br><br>Name: | **Oral test**<br>Acetone<br>**Contact test**<br>Acetone | See Deviations from Guidelines table on page 27 of this draft DER with respect of the absence of the concentration of the pyroxsulam in the acetone solution.<br>*EPA/OECD prefer acetone as a solvent*<br>*EPA: negative and solvent controls required. Positive control not required.* |
| Concentration: | **Oral test:**<br>19-24 mg food (mixed with solvent solutions of pyroxsulam or the toxic standard, or with tap water (water control) or acetone (solvent control)) was consumed by the bees. | In the oral test, the test solutions, solvent (acetone) solutions of pyroxsulam or dimethoate, tap water (water control) or acetone (solvent control) and syrup were mixed together to ensure a final solvent concentration not exceeding 5% (1 part test solution in solvent plus 19 parts syrup). The contaminated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake did not exceed 4 hours for the test item treatments).<br>While OECD 213 states that a 1% concentration of the solvent in the feed is generally appropriate and should not be exceeded, the guideline does recognise that the concentration of the vehicle depends on the solubility of the test substance |

| Parameter | Value | Remarks<br>*Criteria* |
|---|---|---|
| | | The study report stated that the use of a concentration of 5% solvent was reported as essential to obtain the maximum dose rate of 100 µg pyroxsulam/bee. As result, the use of the 5% concentration of the solvent is not considered a deviation from the OECD guideline or a deficiency. |
| | **Contact test:**<br>A single 5 µL droplet of pyroxsulam in acetone.<br><br>For the controls one 5 µL droplet of a) acetone and b) tap water with 1% Adhäsit were used. The toxic standard was applied in 5 µL acetone. | OECD 214 recommends a 1 µL volume but does allow other volumes if justified which was the case in the study report (a higher volume gave more reliable dispersion). The use of the 5 µL droplet is not considered a deviation from the OECD guideline or a deficiency. |
| Number of bees per cage | 10 bees/cage | Requirement considered met.<br>*EPA requires at least 25 bees per treatment*<br>*OECD prefers 10 bees per cage*<br>Because the study was conducted to the OECD guidelines, the use of 10 honeybees per cage is acceptable. |
| Number of cages per treatment | 5 cages (i.e. 5 replicates) per pyroxsulam dose level, water and solvent controls and each toxic standard dose. | OECD requirement met and the US EPA requirement (see following) is exceeded.<br>*EPA: One cage per each treatment level and each control.*<br><br>OECD 213 states a minimum of 3 replicate test groups, each of 10 bees, and a minimum of 3 replicate controls, each of 10 bees, should be used. Where a solvent or a dispersant is used to solubilise the test substance, two separate control groups should be used: a solution in water and a sucrose solution with the solvent/carrier at the concentration used in dosing solutions. |

| Parameter | | Value | Remarks<br>*Criteria* |
|---|---|---|---|
| | | | OECD 214 has similar requirements and notes that if an organic solvent or a wetting agent is used, three additional control batches each of ten bees for the solvent or the wetting agent have to be included. |
| Number of replicates<br><br>Negative control: | | 5 replicates, each consisting of 10 bees in one cage for the water control.<br>5 replicates, each consisting of 10 bees in one cage for the solvent control. | Requirement considered met.<br><br>*OECD requires at least three replicates, each of ten bees*<br>*EPA: Replications are not required.* |
| Treated: | | 5 replicates, each consisting of 10 bees in one cage per test concentration of the toxic standard (dimethoate) at 0.1, 0.15, 0.2 and 0.3 µg/bee in the contact toxicity test and 0.04, 0.08, 0.15 and 0.33 µg/bee in the oral toxicity test.<br>There were 50 bees per treatment group. | |
| For **Acute contact** study | Doses used<br>Nominal:<br><br>Measured: | 100 µg of pyroxsulam/bee (i.e. done as a limit test)<br>No measured dose reported | See deviations from Guidelines table on page 27 of this draft DER.<br>OECD 214 allows for use of a limit test at 100 µg active constituent/bee if the test substance is expected to be of low toxicity – as was indicated by the range finding study's results. US EPA OPPTS 850.3020 also states that a contact toxicity limit test can be conducted if the test substance is expected to be of relatively low toxicity.<br><br>Consequently, the US EPA and OECD requirements with respect to the need for five dosage levels listed in the template are not considered to have been a deviation from the OECD or US EPA guideline.<br><br>*EPA requires at least five dosage levels, spaced geometrically at least 60% of the next higher level*<br>*OECD requires five doses in a geometric series, with a factor not exceeding 2.2* |

| Parameter | | Value | Remarks<br>*Criteria* |
|---|---|---|---|
| | Method of test material application including the body part and volume of test solution applied | A single 5 µL droplet of pyroxsulam in acetone was placed on the ventral bee thorax using a Burkard applicator.<br>For the controls one 5 µL droplet of (a) acetone and (b) tap water with 1 % Adhäsit was used.<br>The toxic standard was applied in 5 µL acetone.<br>The study report stated that Adhäsit was used to improve the adhesion of the droplet on the bee body and that Adhäsit is non-toxic to honey bees.<br>Bees were anaesthetized with carbon dioxide in the contact test | *EPA: Test material administered as single topical dose (topical drop) or whole body exposure to impregnated dust.*<br><br>The study report noted that a 5 µL. droplet was chosen in deviation to the guideline recommendation of 1 µL, since a higher volume ensured a more reliable dispersion of the test item. Ibacon experience was said to have proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected. OECD 214 allows for different volumes of application. |
| | Time of test material application | Not specifically identified but test was indicated as commencing on the day of collection of the bees. | See deviations from Guidelines table on page 27 of this draft DER. |
| For **Acute oral** study | <u>Doses used</u><br><br>Nominal:<br><br>Measured: | 100 µg of pyroxsulam/bee (i.e. done as a limit test)<br>107.4 µg of pyroxsulam/bee (actually a calculated mean based on the treated food source containing 5 µg pyroxsulam/mg food). | See deviations from Guidelines table on page 27 of this draft DER.<br>The study report stated that the dosages applied were adjusted to reflect the analytical percentage of active constituent.<br>OECD 213 allows for use of a limit test at 100 µg active constituent/bee if the test substance is expected to be of low toxicity – as was indicated by the range finding study's results.<br>The study report refers to the measured dosage of the pyroxsulam in the oral test as 107.4 µg/bee, but as noted above, this is a calculated, not analytically determined value.<br>The study report does indicate that the amounts of dimethoate used for the oral and contact tests were "measured" but without presenting analytical details. |
| | Details of the food source | Commercial ready-to-use syrup (Apiinvert; 30% saccharose, 31% glucose, 39% fructose). | |
| Method of feeding during the study | | Commercial ready-to-use syrup (Apiinvert) was given *ad libitum* directly after treatments (applications) | Requirement considered met.<br><br>*EPA: A 50% sugar/water solution* |

| Parameter | Value | Remarks<br>*Criteria* |
|---|---|---|
| | in syringes as a food source for the bees. This untreated syrup (for the contact toxicity testing) was reported as sufficient food during the experimental phase and was not replaced.<br>For the acute oral toxicity test, the syrup (either with pyroxsulam, dimethoate, water or acetone additions) was also provided by means of syringes. | *will be provided ad libitum throughout the holding and test periods. A vial containing solution must be attached to each cage.* |
| Duration of the study | 48 hours for both the oral and contact toxicity studies.<br>Because no mortalities or adverse effects were seen in the pyroxsulam exposed bees, the study was not prolonged beyond this time. | Requirement met with observations taken at 4, 24 and 48 hours.<br>*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 | In the oral test, the starvation time was 15 minutes.<br>The test cages were indicated as ventilated to avoid possible accumulation of pesticide vapours. | Requirement considered met.<br>While OECD 213 states the bees may be starved for up to 2 hours before initiation of the test so that all bees are equal in terms of their gut contents at the start of the test, the 15 minute starvation time is not considered either a deviation from the OECD guideline or a deficiency. |
| Reference chemical, if used<br>Name:<br>Concentration(s): | Dimethoate (as the toxic standard) as the formulated product Perfekthion EC (BAS 152 11 I).<br>Amount applied:<br>Contact test: 0.1-0.3 µg of dimethoate/bee<br>Oral test: 0.04-0.33 µg of dimethoate/bee. | Requirement considered met.<br>OECD 213 and 214 refer to dimethoate as the preferred toxic standard. |

**2. Observations:**

**Table 4. Observations**

<table>
<tr><th rowspan="2">Parameters</th><th rowspan="2">Details</th><th>Remarks</th></tr>
<tr><th>Criteria</th></tr>
<tr><td>Parameters measured including sublethal effects/toxicity symptoms</td><td>Mortality: number of dead bees after 4 hours (first day); 24 and 48 hours.<br><br>Behavioural Abnormalities: behavioural abnormalities (vomiting, apathy, intensive cleaning) after 4 hours (first day); 24 and 48 hours.</td><td>Requirement considered met.<br>With respect to the template requirements of "EPA requires less than 20% mortality in the controls<br>OECD requires less than 10% mortality in the controls" entered in the template under this parameter, the control mortality was 0% (Table 5 refers).</td></tr>
<tr><td>Observation intervals</td><td>4, 24 and 48 hours</td><td>Requirement considered met.<br>EPA /OECD require observation intervals of 4, 24 and 48 h after dosing</td></tr>
<tr><td>Amount of treated diet consumed per group (For acute oral)</td><td>Weight of feed eaten/cage and calculated pyroxsulam intake/bee:<br><table><tr><th>Weight of feed consumed/10 bees, mg</th><th>Pyroxsulam µg/bee</th></tr><tr><td>229</td><td>114.5</td></tr><tr><td>213</td><td>106.5</td></tr><tr><td>210</td><td>105.0</td></tr><tr><td>210</td><td>105.0</td></tr><tr><td>212</td><td>106.0</td></tr></table>Note: feed contains 5 µg pyroxsulam/mg<br><br>Average feed intake as mg/bee:<br><table><tr><th colspan="6">Oral intake of the treated diet as mg diet/bee</th></tr><tr><th>Replicate:</th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th></tr><tr><td>Pyroxsulam 100 µg/bee</td><td>23</td><td>21</td><td>21</td><td>21</td><td>21</td></tr><tr><td>Water control</td><td>23</td><td>23</td><td>23</td><td>22</td><td>24</td></tr><tr><td>Solvent control</td><td>23</td><td>22</td><td>22</td><td>22</td><td>20</td></tr></table></td><td>Requirement considered met.<br>Calculated µg of pyroxsulam or dimethoate consumed/ bee:<br><br>Mean pyroxsulam uptake: 107.4 µg/bee</td></tr>
</table>

| Parameters | Details | Remarks<br>*Criteria* |
|---|---|---|
| | **Average feed intake of feed containing dimethoate as mg feed/bee:**<br><br>**Toxic standard µg dimethoate per bee** / **Replicate No.: 1 2 3 4 5** / **Feed intake, mg/bee**<br>**0.04** 21 22 22 22 23<br>**0.08** 22 22 22 21 22<br>**0.15** 20 19 23 21 21<br>**0.30** 24 20 23 21 21 | |
| Were raw data included? | Not as laboratory records. However, the data contained with the final report was audited in comparison to the raw data and the Quality Assurance Statement confirmed that the final report accurately reflected the raw data.<br>For the periods demanded by the principles of GLP the following documents and materials were to be archived, all raw data, the study protocol, study protocol amendments, a certified copy of the final report, a sample of the test item and of the toxic standard. This is to be fo1lowing the date on which the final report is audited by the Quality Assurance Unit at the Institut für Biologische Analytik und Consulting IBACON GmbH Germany | See deviations from Guidelines table on page 27 of this draft DER with respect to the provision of raw data.<br>OECD 213 and 214 state that raw data for mortality at each dose tested at each observation time must be included in the test report.<br>While the tabulated morality results presented in the study report were identified as "Exact Data", it is not certain that these results were equivalent to the raw laboratory data. Consequently a deviation from the OECD 213 and 214 guidelines is considered to have occurred. |
| Other observations, if any | The study report stated there had been no deviations from the study protocol. | Requirement considered met. |

## II. RESULTS AND DISCUSSION:

### A. MORTALITY:

**Acute oral toxicity**
The cumulative mortality of honey bees exposed to pyroxsulam in the acute oral test is shown in Table 5. There were no mortalities in any of the control or exposed bees. In contrast, exposure to the toxic control, dimethoate, resulted in up to 96% mortality after 48 hours.

**Table 5. Effect of pyroxsulam on cumulative mortality of honey bees in an acute oral test**

| Treatments (nominal µg/bee) – ORAL exposure | No. of bees | Observation period | | | | | |
|---|---|---|---|---|---|---|---|
| | | 4 hours | | 24 hours | | 48 hours | |
| | | No. Dead | % mortality | No. Dead | % mortality | No. Dead | % mortality |
| Negative control (carbon dioxide/water) | 50 | 0 | 0 | 0 | 0 | 0 | 0% |
| Solvent control (Acetone) | 50 | 0 | 0 | 0 | 0 | 0 | 0% |
| Test concentration 1 Nominal 100 µg pyroxsulam/bee calculated mean intake 107.4 µg pyroxsulam/bee | 50 | 0 | 0 | 0 | 0 | 0 | 0% |
| **NOEL/NOEC** | Not reported | | | | | | |
| **$LD_{50}/LC_{50}$** | 48 hour LD50 is >107.4 µg pyroxsulam/bee. | | | | | | |
| **Toxic reference chemical (dimethoate)** | | | | | | | |
| Toxic standard, nominal 0.04 µg/bee | 50 | 0 | 0% | 0 | 0% | 0 | 0% |
| Toxic standard, 0.08 µg/bee | 50 | 0 | 0% | 1 | 2% | 3 | 6% |
| Toxic standard, 0.15 µg/bee | 50 | 0 | 0% | 28 | 56% | 41 | 82% |
| Toxic standard, 0.33 µg/bee | 50 | 2 | 4% | 46 | 92% | 48 | 96% |
| **NOEL/NOEC** | Not reported | | | | | | |
| **$LD_{50}/LC_{50}$** | 24 h LD50 = 0.14 µg dimethoate/bee (95% confidence limits 0.08 and 0.33 µg dimethoate/bee)<br>48 h LD50 = 0.12 µg dimethoate/bee (95% confidence limits 0.08 and 0.15 µg dimethoate/bee) | | | | | | |

Mortalities in the water and solvent control over 48 hours were both 0% in the acute oral test. OECD 213 requires that, for the test to be valid, the average mortality for the total number of controls must not exceed 10 per cent at the end of the test.

This guideline also requires that, to be valid, the reported 24 hour acute LD50 of the toxic standard meets the specified range given in the guideline, namely 0.10-0.35 µg dimethoate/bee. The reported 24 h LD50 of 0.14 µg dimethoate/bee (95% confidence limits 0.08 and 0.33 µg dimethoate/bee) falls within this range, thus meeting the guideline requirement.

As mortality levels in the 107.4 µg pyroxsulam/bee group is below 50%, the oral LD50 was considered by the study report as > 107.4 µg pyroxsulam/bee.

No NOEC was reported by the study report.

**Acute contact toxicity**
The cumulative mortality of honey bees exposed to pyroxsulam in the acute contact test is shown in Table 6. There was one mortality in the control bees between 24 and 48 hours and no mortalities in any of the solvent control or pyroxsulam exposed bees. In contrast, exposure to the toxic control, dimethoate, resulted in up to 98% mortality after 48 hours.

Mortalities in the water and solvent control over 48 hours were respectively, 2 and 0% in the acute oral test. OECD 214 requires that, for the test to be valid, the average mortality for the total number of controls must not exceed 10 per cent at the end of the test.

OECD 214 also requires that, to be valid, the reported contact 24 hour LD50 of the toxic standard meets the specified range given in the guideline, namely, 0.10-0.30 µg dimethoate/bee. The reported 24 h LD50 of 0.14 µg dimethoate/bee (95% confidence limits 0.10 and 0.20 µg dimethoate/bee) falls within this range, thus meeting the guideline requirement with respect to the toxic control LD50.

No NOEC was reported by the study report.

**Table 6. Effect of pyroxsulam on cumulative mortality of honey bees in an acute contact test**

| Treatments (µg pyroxsulam/bee) – CONTACT exposure | No. of bees | Observation period | | | | | |
|---|---|---|---|---|---|---|---|
| | | 4 hours | | 24 hours | | 48 hours | |
| | | No. Dead | % mortality | No. Dead | % mortality | No. Dead | % mortality |
| Negative control (Carbon dioxide/water) | 50 | 0 | 0 | 0 | 0 | 1 | 2% |
| Solvent control (Acetone) | 50 | 0 | 0 | 0 | 0 | 0 | 0% |
| Test concentration 1 nominal 100 µg pyroxsulam/bee | 50 | 0 | 0 | 0 | 0 | 0 | 0% |
| **NOEL/NOEC** | Not reported | | | | | | |
| **$LD_{50}/LC_{50}$** | 48 hour LD50 is >100 µg pyroxsulam/bee. | | | | | | |
| **Toxic reference chemical (dimethoate)** | | | | | | | |
| Toxic standard, 0.10 µg/bee | 50 | 0 | 0% | 1 | 2% | 1 | 2% |
| Toxic standard, 0.15 µg/bee | 50 | 10 | 20% | 30 | 60% | 36 | 72% |
| Toxic standard, 0.20 µg/bee | 50 | 15 | 30% | 42 | 84% | 48 | 96% |
| Toxic standard, 0.30 µg/bee | 50 | 18 | 36% | 47 | 94% | 49 | 98% |
| **NOEL/NOEC** | Not reported | | | | | | |
| **$LD_{50}/LC_{50}$** | 24 h LD50 = 0.14 (95% confidence limits 0.10 and 0.20 µg dimethoate/bee)<br>48 h LD50 = 0.13 µg dimethoate/bee (95% confidence limits 0.10 and 0.15 µg dimethoate/bee) | | | | | | |

Because mortality levels in the 100.0 µg pyroxsulam/bee group were below 50%, the contact LD50 was considered by the study report as >100.0 µg pyroxsulam/bee.

**B. SUB-LETHAL TOXICITY EFFECTS:**

The following tables summarise the behavioural abnormalities recorded in the study report (Table 7 shows the results from the oral exposure while Table 8 details the contact exposure results). There was an absence of sub-lethal toxicity effects (behavioural abnormalities) in both the oral and contact exposure tests with respect to pyroxsulam and the solvent controls over the 48 hours exposure. In contrast, the toxic (positive) control produced movement co-ordination problems and apathy on occasion (see respectively, Table 7 and Table 8).

**Table 7. Effect of pyroxsulam on behaviour of honey bees (oral test).**

| Treatments (µg /bee) | Observation period | | | | | |
|---|---|---|---|---|---|---|
| | After 4 hours | | After 24 hours | | After 48 hours | |
| | endpoint | % affected | endpoint | % effected | endpoint | % effected |
| **Negative (water) control** | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% |
| **Solvent (acetone) control** | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% |
| **107.5 µg pyroxsulam/bee (5 replicates, range 105.0-114.5 µg pyroxsulam/bee).** | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% |
| **NOEC** | Not reported | | | | | |
| **$EC_{50}$ or other sublethal endpoint** | Not reported | | | | | |
| **Toxic reference chemical (dimethoate)** | | | | | | |
| **0.04 µg dimethoate/bee** | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% |
| **0.08 µg dimethoate/bee** | No abnormal behaviour recorded. | 0% | Movement co-ordination problems. | 2% (1 bee affected) | No abnormal behaviour recorded. | 0% |
| **0.15 µg dimethoate/bee** | Movement co-ordination problems. | 4% (2 bees affected in two replicates) | Movement co-ordination problems. | 2% (1 bee affected) | No abnormal behaviour recorded. | 0% |
| **0.33 µg dimethoate/bee** | Movement co-ordination problems and apathy. | 36% (13 bees with both symptoms and 5 with movement problems only. All replicates affected) | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% |
| **$LC_{50}$** | Not reported | | | | | |
| **NOEC** | Not reported | | | | | |

**Table 8. Effect of pyroxsulam on behaviour of honey bees (contact test).**

| Treatments (µg /bee) | Observation period | | | | | |
|---|---|---|---|---|---|---|
| | After 4 hours | | After 24 hours | | After 48 hours | |
| | endpoint | % affected | endpoint | % effected | endpoint | % effected |
| **Negative (water) control** | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% |
| **Solvent (acetone) control** | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% |
| **100 µg pyroxsulam/bee (5 replicates).** | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% |
| **NOEC**<br>**$EC_{50}$ or other sublethal endpoint** | Not reported<br>Not reported | | | | | |
| **Toxic reference chemical (dimethoate)** | | | | | | |
| **0.10 µg dimethoate/bee** | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% | No abnormal behaviour recorded. | 0% |
| **0.15 µg dimethoate/bee** | Movement co-ordination problems. | 22% (11 bees, all replicates affected) | Movement co-ordination problems and apathy. | 12% (5 bees with both symptoms and 1 with apathy only, 3 replicates affected) | No abnormal behaviour recorded. | 0% |
| **0.20 µg dimethoate/bee** | Movement co-ordination problems and apathy. | 46% (15 bees with both symptoms and 8 with apathy only, 5 replicates affected) | Movement co-ordination problems and apathy. | 12% (3 bees with both symptoms and 3 with apathy only. 4 replicates affected) | No abnormal behaviour recorded. | 0% |
| **0.30 µg dimethoate/bee** | Movement co- | 58% (19 bees with | Apathy | 6% (1 bee in one | Apathy | 2% (1 bee in one |

| Treatments (µg /bee) | Observation period | | | | | |
|---|---|---|---|---|---|---|
| | After 4 hours | | After 24 hours | | After 48 hours | |
| | endpoint | % affected | endpoint | % effected | endpoint | % effected |
| | ordination problems and apathy | both symptoms and 10 with apathy only. 5 replicates affected) | | replicate and 2 bees in a second replicate affected. | | replicate affected) |
| $LC_{50}$<br>NOEC | Not reported<br>Not reported | | | | | |

**Consumption of control, pyroxsulam and dimethoate treated diets**

The consumption of treated diet in the pyroxsulam, control and dimethoate solutions are shown in Table 9, page 23 of this draft DER (means determined by the reviewer).

The reviewer's statistical analysis of these results (*vide infra*) indicate that the amounts of treated diet consumed by the bees exposed to the pyroxsulam containing syrup were not statistically significantly different from the amounts consumed by the water and solvent controls. The results from the 0.15 µg dimethoate/bee treatment are identified as significantly lower that the pooled controls but this is considered irrelevant in relation to the amounts of pyroxsulam containing syrup consumed (see Verification of Statistical Results below).

**C. REPORTED STATISTICS:**

Results obtained from the bees treated with test item were compared to those obtained from the toxic standard and the controls. The contact and oral LD50s of the toxic standard were estimated using the binomial distribution (according to Stephan, 1977).

The LD50 calculations were conducted taking into account the mortality data corrected by control mortality using Abbott's formula (1925).

The software used to perform the statistical analysis was ToxRat Professional, Version 2.07, ® ToxRat Solutions GmbH,© 2001-2003.

The statistical calculations were not presented in the study report. However, OECD 213 and 214 only refer to the need to have the statistical procedures used in the determination of the LD50 presented in the study report and the lack of statistical calculations in the report is not considered a deviation from the guidelines.

**D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:**

The statistical evaluation of the consumption of treated diet in the pyroxsulam, control and dimethoate solutions was conducted by the reviewer. The toxic standard's LD50 values were also re-calculated from the survival data presented in the study report. Statistical analyses were conducted with the TidePool Scientific Software ToxCalc™ v5.0.0.23j Environmental Toxicity Data Analysis package.

**Dietary intakes**

The amounts of the commercial syrup, as mg/bee, eaten by the bees in the oral toxicity test were reported as shown in Table 9 with the means calculated by the reviewer.

**Table 9. Calculated amounts of control and treated syrups eaten by honey bees.**

| | Amounts of syrup eaten in the oral toxicity test (mg syrup/bee) | | | | | Mean values mg syrup/bee |
|---|---|---|---|---|---|---|
| **Replicate number:** | **1** | **2** | **3** | **4** | **5** | |
| **Water control** | 23 | 23 | 23 | 22 | 24 | 23.0 |
| **Solvent control** | 23 | 22 | 22 | 22 | 20 | 21.8 |
| **Pyroxsulam** | 23 | 21 | 21 | 21 | 21 | 21.4 |
| **Toxic standard (µg dimethoate/bee)** | | | | | | |
| **0.04** | 21 | 22 | 22 | 22 | 23 | 22.0 |
| **0.08** | 22 | 22 | 22 | 21 | 22 | 21.8 |
| **0.15** | 20 | 19 | 23 | 21 | 21 | 20.8 |
| **0.36** | 24 | 20 | 23 | 21 | 21 | 21.8 |

The ToxCalc analysis of this data (mg of syrup eaten/bee (without transformation)) reported a normal distribution (Shapiro-Wilk's Test, $p > 0.01$). Bartlett's Test indicated that the variances were equal ($p = 0.06$). The water and solvent control means were not significantly different ($p = 0.07$) and were pooled. Compared to the pooled mean, only the 0.15 mg dimethoate/L mean was statistically significantly different (t-test). The following ToxCalc printout summarises these results.

| | | | Transform: Untransformed | | | | | | 1-Tailed | |
|---|---|---|---|---|---|---|---|---|---|---|
| **ug/bee** | **Mean** | **N-Mean** | **Mean** | **Min** | **Max** | **CV%** | **N** | **t-Stat** | **Critical** | **MSD** |
| Pooled | 22.400 | 1.0000 | 22.400 | 20.000 | 24.000 | 4.799 | 10 | | | |
| 0.04 | 22.000 | 0.9821 | 22.000 | 21.000 | 23.000 | 3.214 | 5 | 0.655 | 2.462 | 1.502 |
| 0.08 | 21.800 | 0.9732 | 21.800 | 21.000 | 22.000 | 2.051 | 5 | 0.983 | 2.462 | 1.502 |
| *0.15 | 20.800 | 0.9286 | 20.800 | 19.000 | 23.000 | 7.131 | 5 | 2.622 | 2.462 | 1.502 |
| 0.3 | 21.800 | 0.9732 | 21.800 | 20.000 | 24.000 | 7.537 | 5 | 0.983 | 2.462 | 1.502 |
| 100 | 21.400 | 0.9554 | 21.400 | 21.000 | 23.000 | 4.180 | 5 | 1.639 | 2.462 | 1.502 |

| **Auxiliary Tests** | **Statistic** | **Critical** | **Skew** | **Kurt** |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$) | 0.96103 | 0.91 | 0.11682 | 0.49025 |
| Bartlett's Test indicates equal variances ($p = 0.20$) | 7.29972 | 15.0863 | | |
| The control means are not significantly different ($p = 0.07$) | 2.05798 | 2.306 | | |

These results indicate that the amounts of treated diet consumed by the bees exposed to the pyroxsulam containing syrup were not statistically significantly different from the amounts consumed by the water and solvent controls. The 0.15 µg dimethoate/bee result is identified as significantly lower that the pooled controls but this is considered unlikely to be of biological significance as the effect was not seen to be dose related.

**Dimethoate LD50 values**

**Acute oral toxicity associated with dimethoate exposure**

The numbers of bees alive/replicate at the start, at 24 and 48 hours in the oral acute toxicity test are shown in Table 10.

**Table 10. Number of bees alive at 0, 24 and 48 hours following acute oral exposure to a dimethoate treated syrup.**

| Test Sample | Replicate No. | Number of bees alive at: 0 hours | 24 hours | 48 hours |
|---|---|---|---|---|
| Water control | 1 | 10 | 10 | 10 |
| | 2 | 10 | 10 | 10 |
| | 3 | 10 | 10 | 10 |
| | 4 | 10 | 10 | 10 |
| | 5 | 10 | 10 | 10 |
| Solvent control | 1 | 10 | 10 | 10 |
| | 2 | 10 | 10 | 10 |
| | 3 | 10 | 10 | 10 |
| | 4 | 10 | 10 | 10 |
| | 5 | 10 | 10 | 10 |
| 0.04 µg dimethoate/bee | 1 | 10 | 10 | 10 |
| | 2 | 10 | 10 | 10 |
| | 3 | 10 | 10 | 10 |
| | 4 | 10 | 10 | 10 |
| | 5 | 10 | 10 | 10 |
| 0.08 µg dimethoate/bee | 1 | 10 | 10 | 9 |
| | 2 | 10 | 10 | 10 |
| | 3 | 10 | 9 | 9 |
| | 4 | 10 | 10 | 10 |
| | 5 | 10 | 10 | 9 |
| 0.15 µg dimethoate/bee | 1 | 10 | 6 | 0 |
| | 2 | 10 | 3 | 1 |
| | 3 | 10 | 1 | 0 |
| | 4 | 10 | 6 | 4 |
| | 5 | 10 | 6 | 4 |
| 0.30 µg dimethoate/bee | 1 | 10 | 0 | 0 |
| | 2 | 10 | 3 | 1 |
| | 3 | 10 | 1 | 1 |
| | 4 | 10 | 0 | 0 |
| | 5 | 10 | 0 | 0 |

**Survival after 24 hours exposure (oral) to dimethoate**

The ToxCalc analysis of the bees alive at 24 hours data (with arc sine square root transformation of the data) reported a non-normal distribution (Shapiro-Wilk's Test, $p \leq 0.01$) and equality of variances could not be confirmed. The water and solvent control means were not significantly different ($p = 1.00$) and were pooled. The 24 hour LD50 was calculated using the ToxCalc maximum likelihood probit as 0.15 µg dimethoate/bee with 95% fiducial limits of 0.14 and 0.17 µg dimethoate/bee.

A summary of the ToxCalc results for the 24 hour dimethoate survival results is provided on page 32 of this draft DER.

**Survival after 48 hours exposure (oral) to dimethoate**
The ToxCalc analysis of the bees alive at 48 hours (with arc sine square root transformation of the data) reported a non-normal distribution (Shapiro-Wilk's Test, $p \leq 0.01$) and equality of variances could not be confirmed. The water and solvent control means were not significantly different ($p = 1.00$) and were pooled. The 48 hour LD50 was calculated using the ToxCalc maximum likelihood logit function as 0.12 µg dimethoate/bee with 95% fiducial limits of 0.09 and 0.16 dimethoate/bee.

Summaries of the ToxCalc results for the 24 and 48 hour oral toxicity survival results after dimethoate exposure are provided on, respectively, pages 32 and 33 of this draft DER.

**Acute contact toxicity associated with dimethoate exposure**
The numbers of bees alive/replicate at the start, at 24 and 48 hours in the oral acute toxicity test reported are shown in Table 11.

**Table 11. Numbers of honey bees alive at 0, 24 and 48 hours after acute contact exposure to dimethoate.**

| Test Sample | Replicate No. | Number of bees alive at: 0 hours | 24 hours | 48 hours |
|---|---|---|---|---|
| **Water control** | **1** | 10 | 10 | 10 |
| | **2** | 10 | 10 | 9 |
| | **3** | 10 | 10 | 10 |
| | **4** | 10 | 10 | 10 |
| | **5** | 10 | 10 | 10 |
| **Solvent control** | **1** | 10 | 10 | 10 |
| | **2** | 10 | 10 | 10 |
| | **3** | 10 | 10 | 10 |
| | **4** | 10 | 10 | 10 |
| | **5** | 10 | 10 | 10 |
| **0.10 µg/bee** | **1** | 10 | 10 | 10 |
| | **2** | 10 | 10 | 10 |
| | **3** | 10 | 10 | 10 |
| | **4** | 10 | 10 | 10 |
| | **5** | 10 | 9 | 9 |
| **0.15 µg/bee** | **1** | 10 | 4 | 3 |
| | **2** | 10 | 3 | 2 |
| | **3** | 10 | 4 | 3 |
| | **4** | 10 | 5 | 2 |
| | **5** | 10 | 4 | 4 |
| **0.20 µg/bee** | **1** | 10 | 0 | 0 |
| | **2** | 10 | 2 | 1 |
| | **3** | 10 | 3 | 0 |
| | **4** | 10 | 1 | 0 |
| | **5** | 10 | 2 | 1 |
| **0.30 µg/bee** | **1** | 10 | 0 | 0 |
| | **2** | 10 | 1 | 0 |
| | **3** | 10 | 2 | 1 |
| | **4** | 10 | 0 | 0 |
| | **5** | 10 | 0 | 0 |

**Survival after 24 hours exposure (contact) with dimethoate**
The ToxCalc analysis of the bees alive at 24 hours data (with arc sine square root transformation of the data) reported a normal distribution (Shapiro-Wilk's Test, $p > 0.01$) but equality of variances could not be confirmed. The water and solvent control means were not significantly different ($p = 1.00$) and were pooled. The 24 hour LD50 was calculated using the ToxCalc maximum likelihood logit function as 0.15 µg dimethoate/bee with 95% fiducial limits of 0.12 and 0.18 µg dimethoate/bee.

A summary of the ToxCalc results for the 24 hour dimethoate survival results is provided on page 34 of this draft DER.

**Survival after 48 hours exposure (contact) with dimethoate**
The ToxCalc analysis of the bees alive at 48 hours data (with arc sine square root transformation of the data) reported a normal distribution (Shapiro-Wilk's Test, $p > 0.01$) while Bartlett's Test indicated equality of variances ($0 = 0.65$). The water and solvent control means were not significantly different ($p = 1.00$) and were pooled. The 48 hour LD50 was calculated using the ToxCalc maximum likelihood angular procedure as 0.14 µg dimethoate/bee with 95% fiducial limits of 0.08 and 0.18 µg dimethoate/bee.

A summary of the ToxCalc results for the 48 hour survival results after dimethoate exposure is provided on page 35 of this draft DER.

The reviewer calculated dimethoate endpoints, reported endpoint values and OECD recommendations are summarised in Table 12.

**Table 12. Reviewer calculated dimethoate endpoints, reported dimethoate endpoint values and OECD recommendations for dimethoate endpoints.**

| Acute oral toxicity | Study report value | Reviewer calculated value | OECD reported range for the 24 hour dimethoate LD50 |
|---|---|---|---|
| **24 hour LD50** | 0.14 (0.08-0.33) µg/bee | 0.15 (0.14-0.17) µg/bee | 0.10-0.35 µg/bee (OECD 213) |
| **48 hour LD50** | 0.12 (0.08-0.15) µg/bee | 0.12 (0.09-0.16) µg/bee | |
| **Acute contact toxicity** | | | |
| **24 hour LD50** | 0.14 (0.10-0.20) µg/bee | 0.15 (0.12-0.18) µg/bee | 0.10-0.30 µg/bee (OECD 214) |
| **48 hour LD50** | 0.13 (0.10-0.15) µg/bee | 0.14 (0.08-0.18) µg/bee | |

The reviewer calculated statistics are considered to have verified the relevant applicant's results with respect to feed consumption of the bees treated by the oral exposure route and the LD50s reported for dimethoate.

**E. STUDY DEFICIENCIES:**

The following table identifies deviations from OECD 213 and OECD 214 and other deficiencies remarked upon in the preparation of this DER.

**Table 13. Deviations from guidelines and other study deficiencies**

| Parameter | Study reported results | Guideline value/comment |
|---|---|---|
| Stability of Compound under Test Conditions | Stability in acetone (and water) not reported. | The absence of information in the study report on the stability of pyroxsulam in water and acetone solutions is a deficiency which would be significant if hydrolysis or degradation were an issue. The data seen to date in the evaluation of the study reports for fish and aquatic invertebrates indicates pyroxsulam is stable in aqueous and acetone solutions.<br><br>The study to investigate hydrolysis of pyroxsulam (Yoder, 2005) was conducted in the dark at 20 °C in sterile aqueous buffered solutions at pH 5 (sodium acetate buffer), pH 7 (TRIS buffer) and pH 9 (sodium tetraborate buffer) for 32 days. The study's results showed that pyroxsulam was stable to hydrolysis.<br><br>However, as OECD 213 and 214 state that relevant physico-chemical properties must be included in the test report, the absence of the stability data is a deviation from the guidelines. |
| Cultural Background | It was not indicated that the bees were kept to standard practices as required by the template | OECD 213 and 214 state that all relevant information on colonies used for collection of test bees, including health, any adult disease, any pre-treatment, etc. must be included in the test report. Consequently, the lack of this information is a deviation from the guidelines. |
| Solvent/dispersant control, if used | | The preparation of pyroxsulam in the water control and acetone solutions was not described and the concentration of the pyroxsulam in the acetone solution was not identified. |
| Concentration: | **Oral test:**<br>The test solutions and syrup were mixed together to ensure a final solvent concentration not exceeding 5%. | OECD 213 does not require measurement of the test doses. |
| | **Contact test:**<br>A single 5 µL droplet of pyroxsulam in acetone. | The preparation of the acetone/pyroxsulam test solution was not described nor was the actual concentration used identified.<br><br>OECD 214 does not require measurement of the test doses. |
| Time of test material application – acute contact toxicity | Not specifically identified but test indicated as commenced on the day of collection of the bees. | Absence of this information not considered to have affected the validity of the study or to have adversely affected its results. |
| For **Acute oral** study <u>Doses used</u> | 107.4 µg of pyroxsulam/bee referred to. | The study report refers to the measured dosage of the pyroxsulam in the oral test as 107.4 µg/bee, but this is a calculated, not |

| | | |
|---|---|---|
| | | analytically determined value.<br>The study report does indicate that the amounts of dimethoate used for the oral and contact tests were "measured" but without presenting analytical details.<br><br>The concentration of pyroxsulam in acetone was not reported; reference is only made to mixing the "test solution" with syrup so that the final solvent concentration did not exceed 5% (1 part test solution and 19 parts syrup). |
| For **Acute contact** study<br>Doses used | A single 5 µL droplet of pyroxsulam in acetone was placed on the ventral bee thorax. | The concentration of the pyroxsulam in acetone solution was not stated in the study report.<br>Use of carbon dioxide to anesthetise the bees was indicated but not described. |
| Were raw data included? | Laboratory records not provided. Tabulated mortality and behavioural abnormalities data were provided and identified as "Exact Data". | OECD 213 and 214 state that raw data for mortality at each dose tested at each observation time must be included in the test report.<br>While the tabulated morality results presented in the study report were identified as "Exact Data", it is not certain that these results were equivalent to the raw laboratory data. Consequently, a deviation from the OECD 213 and 214 guidelines could be interpreted as having occurred.<br><br>However, the tabulated data presented were sufficient to allow statistical verification of the study's results and, consequently, the absence of raw data is not considered to have adversely affected the reviewer's assessment of the study.<br><br>The US EPA advised that, tabular data are usually considered "raw data" with the guiding principle being whether the data presented allowed repeating of the statistical analyses. This is considered to support the decision that the raw data absence was not of significance on this occasion. |
| OECD 213 and 214 requirement | OECD 213 and 214 state that, *inter alia*, the structural formula of the active ingredient must be provided. | The pyroxsulam structure was not provided, but this is considered of minor import with respect to the current work share program. |

While it may be assumed that the correct dosages of pyroxsulam and dimethoate in the acetone solutions were prepared for the contact test and for dosing the syrup in the acute toxicity test, the absence of evidence to this end leads to the conclusion that the study should be regarded as supplemental. However, OECD 213 and 214 do not require such information be presented, with this resulting in the study being regarded as acceptable. Other deficiencies and deviations identified are not considered to have invalidated either the study or its results.

**F. REVIEWER'S COMMENTS:**

The study's results meet the validity requirements of both OECD 213 and 214 with respect to the average mortality for the total number of controls not exceeding 10% at the end of the test and the acute and contact LD50s for the toxic standard being within the OECD specified ranges.

The absence of toxicity of pyroxsulam to the bees in the oral and contact toxicity testing shows pyroxsulam would be classified as very slightly toxic to the honey bee in accordance with the classification system of the Australian Government Department of the Environment and Water Resources or as practically non-toxic according to the US EPA ecotoxicity categories. However, the lack of information on the concentration of pyroxsulam in the acetone solution used for the contact toxicity test and for making up the treated syrup solution for the oral toxicity test is a deficiency. Based on the GLP compliance and quality assurance unit statements provided, the reviewer believes this information could be obtained from the archived documentation. Because OECD 213 and 214 do not specify that such details must be provided, the study has been classified as "Acceptable" rather than "Supplemental".

The study report did not establish NOEC values. The reviewer considers NOECs for contact toxicity mortality and sublethal effects should be set at 100 µg pyroxsulam/bee and, for oral toxicity, at 107.4 µg pyroxsulam/bee.

The experimental starting date was 3 September 2003 and its completion date, 12 September 2003.

The PMRA agrees with the conclusions of the study author and of the APVMA reviewer.

## G. CONCLUSIONS:

This study is classified as acceptable to DEW and the PMRA and considered to satisfy the guideline requirements for acute contact toxicity and acute oral toxicity studies for honey bees. For the US EPA, the honey bee contact acute toxicity test is classified as acceptable. Because the honey bee oral toxicity test is not required by EPA, this component of the study is classified supplemental.

| **For acute contact** | | **For acute oral** | |
|---|---|---|---|
| LD50: | >100 µg pyroxsulam/bee | LC50: | >107.4 µg pyroxsulam/bee |
| Confidence Interval: | Not applicable | Confidence Interval: | Not applicable |
| Slope: | Not applicable | Slope: | Not applicable |
| NOEL: | Not reported but set at 100 µg pyroxsulam/bee by the reviewer. | NOEC: | Not reported but set at 107.4 µg pyroxsulam/bee by the reviewer. |

Based on the results of this study, pyroxsulam would be classified as very slightly toxic to the honey bee (LC50 and LD50 >100 µg/bee) in accordance with the classification system of the Australian Government Department of the Environment and Water Resources or as practically non-toxic according to the US EPA ecotoxicity categories (http://www.epa.gov/oppefed1/ecorisk_ders/toera_analysis_eco.htm#Ecotox) (LD50 >11 µg/bee) – taken as equivalent to the toxicity ratings of Atkins (1981).

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Mercer J (2006b) Study Profile Template (SPT) for Effects of XDE-742/BAS770H Acute oral on Honey Bees (*Apis mellifera* L.) in the laboratory. Institute fur biologische analytic und consulting IBACON GmbH, Arheilger Weg 17 64380 Rossdorf Germany. IBACON study number: 18361035.SPT. The Dow Chemical

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Approved 04/01/01 C.K.

**Attachment 1**
**Dimethoate oral toxicity – 24 hour toxicity**

The numbers of bees surviving at 24 hours are discussed on page 24 of this draft DER.

ToxCalc analysis of the numbers of bees surviving after 24 hours and oral exposure to dimethoate in syrup fed to the bees:

**Fraction of bees surviving after 24 hours (1 = 100% survival):**

| Conc-µg/bee | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| B-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| S-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 0.04 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 0.08 | 1.0000 | 1.0000 | 0.9000 | 1.0000 | 1.0000 |
| 0.15 | 0.6000 | 0.3000 | 0.1000 | 0.6000 | 0.6000 |
| 0.3 | 0.0000 | 0.3000 | 0.1000 | 0.0000 | 0.0000 |

**ToxCalc treatment of the data and results:**

| | | | Transform: Arcsin Square Root | | | | | Number | Total |
|---|---|---|---|---|---|---|---|---|---|
| Conc-µg/bee | Mean | N-Mean | Mean | Min | Max | CV% | N | Resp | Number |
| Pooled | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 10 | 0 | 100 |
| 0.04 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 5 | 0 | 50 |
| 0.08 | 0.9800 | 0.9800 | 1.3794 | 1.2490 | 1.4120 | 5.284 | 5 | 1 | 50 |
| 0.15 | 0.4400 | 0.4400 | 0.7119 | 0.3218 | 0.8861 | 35.861 | 5 | 28 | 50 |
| 0.3 | 0.0800 | 0.0800 | 0.2755 | 0.1588 | 0.5796 | 66.798 | 5 | 46 | 50 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.83541 | 0.9 | -0.5241 | 3.9594 |
| Equality of variance cannot be confirmed | | | | |
| The control means are not significantly different (p = 1.00) | 0 | 2.306 | | |

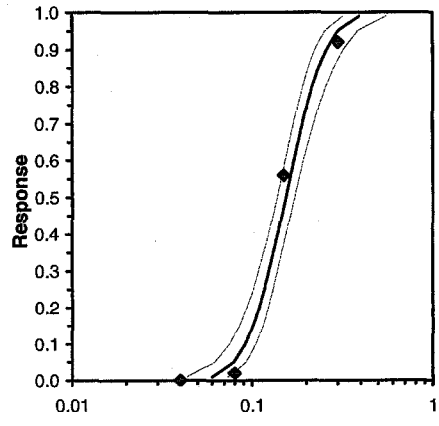
Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 5.69882 | 0.71402 | 4.29933 | 7.09831 | 0 | 3.55676 | 5.99146 | 0.17 | -0.8124 | 0.17547 | 3 |

Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 5.69882 | 0.71402 | 4.29933 | 7.09831 | 0 | 3.55676 | 5.99146 | 0.17 | -0.8124 | 0.17547 | 3 |
| Intercept | 9.62944 | 0.60317 | 8.44723 | 10.8117 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 0.06018 | 0.04361 | 0.07384 |
| EC05 | 3.355 | 0.07925 | 0.06226 | 0.09294 |
| EC10 | 3.718 | 0.09178 | 0.07505 | 0.10536 |
| EC15 | 3.964 | 0.10134 | 0.08497 | 0.11491 |
| EC20 | 4.158 | 0.10964 | 0.09362 | 0.12331 |
| EC25 | 4.326 | 0.1173 | 0.10159 | 0.13121 |
| EC40 | 4.747 | 0.13906 | 0.12368 | 0.1548 |
| EC50 | 5.000 | 0.15405 | 0.13814 | 0.17234 |
| EC60 | 5.253 | 0.17065 | 0.15331 | 0.19308 |
| EC75 | 5.674 | 0.2023 | 0.18016 | 0.23602 |
| EC80 | 5.842 | 0.21644 | 0.19148 | 0.25638 |
| EC85 | 6.036 | 0.23416 | 0.20528 | 0.28277 |
| EC90 | 6.282 | 0.25854 | 0.22366 | 0.32044 |
| EC95 | 6.645 | 0.29942 | 0.25334 | 0.38664 |
| EC99 | 7.326 | 0.39433 | 0.31856 | 0.5525 |



**Attachment 2**
**Dimethoate oral toxicity – 48 hours toxicity**
The numbers of bees surviving at 48 hours are discussed on page 25 of this draft DER.

ToxCalc analysis of the numbers of bees surviving after 24 hours and oral exposure to dimethoate in syrup fed to the bees:

**Fraction of bees surviving after 48 hours oral exposure (1 = 100% survival):**

| Conc-µg/bee | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| B-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| S-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 0.04 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 0.08 | 0.9000 | 1.0000 | 0.9000 | 1.0000 | 0.9000 |
| 0.15 | 0.0000 | 0.1000 | 0.0000 | 0.4000 | 0.4000 |
| 0.3 | 0.0000 | 0.1000 | 0.1000 | 0.0000 | 0.0000 |

**ToxCalc treatment of the data and results:**

| | | | Transform: Arcsin Square Root | | | | | Number | Total |
|---|---|---|---|---|---|---|---|---|---|
| Conc-µg/bee | Mean | N-Mean | Mean | Min | Max | CV% | N | Resp | Number |
| Pooled | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 10 | 0 | 100 |
| 0.04 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 5 | 0 | 50 |
| 0.08 | 0.9400 | 0.9400 | 1.3142 | 1.2490 | 1.4120 | 6.792 | 5 | 3 | 50 |
| 0.15 | 0.1800 | 0.1800 | 0.4017 | 0.1588 | 0.6847 | 66.396 | 5 | 41 | 50 |
| 0.3 | 0.0400 | 0.0400 | 0.2240 | 0.1588 | 0.3218 | 39.855 | 5 | 48 | 50 |

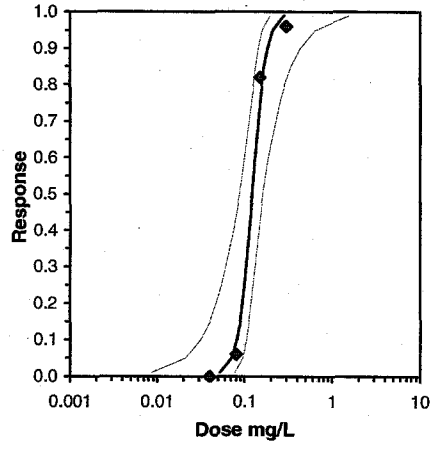
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.83322 | 0.9 | 0.51058 | 2.63284 |
| Equality of variance cannot be confirmed | | | | |
| The control means are not significantly different (p = 1.00) | 0 | 2.306 | | |

**Maximum Likelihood-Logit**

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 12.5277 | 1.95255 | 4.12658 | 20.9289 | 0 | 9.01204 | 5.99146 | 1.0E-02 | | | 10 |
| Intercept | 11.4413 | 1.78387 | 3.76597 | 19.1167 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Logits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | -4.595 | 0.05247 | 0.00854 | 0.07766 |
| EC05 | -2.944 | 0.07107 | 0.02101 | 0.09511 |
| EC10 | -2.197 | 0.08153 | 0.03131 | 0.10512 |
| EC15 | -1.735 | 0.08877 | 0.03987 | 0.11246 |
| EC20 | -1.386 | 0.09464 | 0.0476 | 0.11886 |
| EC25 | -1.099 | 0.09978 | 0.05487 | 0.12496 |
| EC40 | -0.405 | 0.11333 | 0.07524 | 0.14479 |
| EC50 | 0.000 | 0.1221 | 0.08809 | 0.16215 |
| EC60 | 0.405 | 0.13155 | 0.10046 | 0.18641 |
| EC75 | 1.099 | 0.14942 | 0.11899 | 0.25008 |
| EC80 | 1.386 | 0.15754 | 0.12578 | 0.28669 |
| EC85 | 1.735 | 0.16795 | 0.13356 | 0.34071 |
| EC90 | 2.197 | 0.18286 | 0.14346 | 0.43206 |
| EC95 | 2.944 | 0.20977 | 0.15914 | 0.64161 |
| EC99 | 4.595 | 0.28413 | 0.19554 | 1.57291 |

Significant heterogeneity detected (p = 1.00E-02)



**Attachment 3**
**Dimethoate contact toxicity – 24 hours**
The numbers of bees surviving at 24 hours are discussed on page 26 of this draft DER.

ToxCalc analysis of the numbers of bees surviving after 24 hours contact to exposure to dimethoate:

**Fraction of bees surviving after 24 hours contact exposure (1 = 100% survival):**

| Conc-µg/bee | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| D-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| S-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 0.1 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 0.9000 |
| 0.15 | 0.4000 | 0.3000 | 0.4000 | 0.5000 | 0.4000 |
| 0.2 | 0.0000 | 0.2000 | 0.3000 | 0.1000 | 0.2000 |
| 0.3 | 0.0000 | 0.1000 | 0.2000 | 0.0000 | 0.0000 |

**ToxCalc treatment of the data and results:**

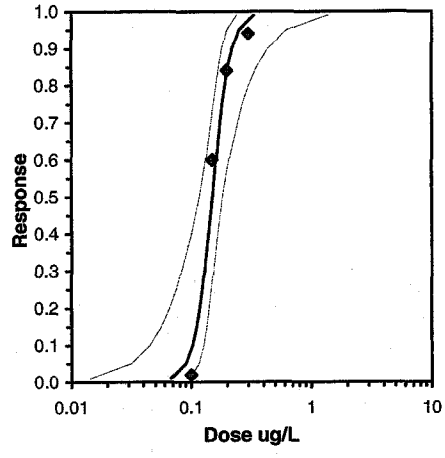
| | | | Transform: Arcsin Square Root | | | | | Number | Total |
|---|---|---|---|---|---|---|---|---|---|
| Conc-µg/bee | Mean | N-Mean | Mean | Min | Max | CV% | N | Resp | Number |
| Pooled | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 10 | 0 | 100 |
| 0.1 | 0.9800 | 0.9800 | 1.3794 | 1.2490 | 1.4120 | 5.284 | 5 | 1 | 50 |
| 0.15 | 0.4000 | 0.4000 | 0.6838 | 0.5796 | 0.7854 | 10.639 | 5 | 30 | 50 |
| 0.2 | 0.1600 | 0.1600 | 0.3975 | 0.1588 | 0.5796 | 40.692 | 5 | 42 | 50 |
| 0.3 | 0.0600 | 0.0600 | 0.2523 | 0.1588 | 0.4636 | 54.526 | 5 | 47 | 50 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.91355 | 0.9 | -0.1243 | 1.8251 |
| Equality of variance cannot be confirmed | | | | |
| The control means are not significantly different (p = 1.00) | 0 | 2.306 | | |

| | | | Maximum Likelihood-Logit | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
| Slope | 13.1948 | 1.98044 | 4.67363 | 21.7159 | 0 | 9.63829 | 5.99146 | 8.1E-03 | | | 9 |
| Intercept | 10.8508 | 1.61178 | 3.91583 | 17.7857 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Logits | ug/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | -4.595 | 0.06751 | 0.01424 | 0.09627 |
| EC05 | -2.944 | 0.09005 | 0.03172 | 0.11615 |
| EC10 | -2.197 | 0.10259 | 0.04535 | 0.12709 |
| EC15 | -1.735 | 0.11122 | 0.05638 | 0.13484 |
| EC20 | -1.386 | 0.11819 | 0.06623 | 0.1414 |
| EC25 | -1.099 | 0.12428 | 0.07542 | 0.14751 |
| EC40 | -0.405 | 0.14025 | 0.10111 | 0.16662 |
| EC50 | 0.000 | 0.15054 | 0.11726 | 0.18315 |
| EC60 | 0.405 | 0.16158 | 0.1325 | 0.20661 |
| EC75 | 1.099 | 0.18235 | 0.15423 | 0.2688 |
| EC80 | 1.386 | 0.19174 | 0.16196 | 0.3041 |
| EC85 | 1.735 | 0.20375 | 0.17075 | 0.35532 |
| EC90 | 2.197 | 0.22089 | 0.18195 | 0.43986 |
| EC95 | 2.944 | 0.25165 | 0.19984 | 0.62644 |
| EC99 | 4.595 | 0.33566 | 0.24185 | 1.39064 |

Significant heterogeneity detected (p = 8.07E-03)



**Attachment 4**
**Dimethoate contact toxicity – 48 hours**
The numbers of bees surviving at 48 hours are discussed on page 26 of this draft DER.

ToxCalc analysis of the numbers of bees surviving after 24 hours contact to exposure to dimethoate:

**Fraction of bees surviving after 24 hours contact exposure (1 = 100% survival):**

| Conc-µg/bee | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| B-Control | 1.0000 | 0.9000 | 1.0000 | 1.0000 | 1.0000 |
| S-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 0.1 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 0.9000 |
| 0.15 | 0.3000 | 0.2000 | 0.3000 | 0.2000 | 0.4000 |
| 0.2 | 0.0000 | 0.1000 | 0.0000 | 0.0000 | 0.1000 |
| 0.3 | 0.0000 | 0.0000 | 0.1000 | 0.0000 | 0.0000 |

**ToxCalc treatment of the data and results:**

| | | | Transform: Arcsin Square Root | | | | | Number | Total |
|---|---|---|---|---|---|---|---|---|---|
| Conc-µg/bee | Mean | N-Mean | Mean | Min | Max | CV% | N | Resp | Number |
| Pooled | 0.9900 | 1.0000 | 1.3957 | 1.2490 | 1.4120 | 3.692 | 10 | 1 | 100 |
| 0.1 | 0.9800 | 0.9899 | 1.3794 | 1.2490 | 1.4120 | 5.284 | 5 | 1 | 50 |
| 0.15 | 0.2800 | 0.2828 | 0.5543 | 0.4636 | 0.6847 | 16.811 | 5 | 36 | 50 |
| 0.2 | 0.0400 | 0.0404 | 0.2240 | 0.1588 | 0.3218 | 39.855 | 5 | 48 | 50 |
| 0.3 | 0.0200 | 0.0202 | 0.1914 | 0.1588 | 0.3218 | 38.084 | 5 | 49 | 50 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.93372 | 0.9 | -0.1561 | 0.11685 |
| Bartlett's Test indicates equal variances (p = 0.65) | 2.46874 | 13.2767 | | |
| The control means are not significantly different (p = 0.35) | 1 | 2.306 | | |

Maximum Likelihood-Angular

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 3.25778 | 0.47718 | 1.20462 | 5.31093 | 0.01 | 10.2831 | 5.99146 | 5.9E-03 | | | 4 |
| Intercept | 3.54957 | 0.37011 | 1.9571 | 5.14203 | | | | | | | |
| TSCR | 0.00821 | 0.02043 | -0.0797 | 0.09613 | | | | | | | |

| Point | Radians | ug/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 0.100 | 0.08733 | 0.02458 | 0.12263 |
| EC05 | 0.226 | 0.09542 | 0.03097 | 0.1306 |
| EC10 | 0.322 | 0.10214 | 0.03692 | 0.1373 |
| EC15 | 0.398 | 0.10777 | 0.04235 | 0.14301 |
| EC20 | 0.464 | 0.11292 | 0.04766 | 0.14833 |
| EC25 | 0.524 | 0.1178 | 0.053 | 0.15351 |
| EC40 | 0.685 | 0.13201 | 0.06998 | 0.16965 |
| EC50 | 0.785 | 0.14175 | 0.08258 | 0.18206 |
| EC60 | 0.886 | 0.1522 | 0.09654 | 0.19721 |
| EC75 | 1.047 | 0.17056 | 0.1206 | 0.23033 |
| EC80 | 1.107 | 0.17794 | 0.12964 | 0.24662 |
| EC85 | 1.173 | 0.18643 | 0.13939 | 0.26773 |
| EC90 | 1.249 | 0.19671 | 0.15022 | 0.29685 |
| EC95 | 1.345 | 0.21056 | 0.16325 | 0.34231 |
| EC99 | 1.471 | 0.23007 | 0.17922 | 0.41835 |

Significant heterogeneity detected (p = 5.85E-03)