

## Final Report

# Effects of Imidacloprid SL 200 (Acute Contact and Oral LD<sub>50</sub>) on Honey Bees (*Apis mellifera* L.) in the Laboratory

(GLP compliant study based on OECD 213 and 214 (1998)  
and the recent recommendations of the ICPBR group, held  
in Avignon, France, 1999)

Author: Dipl. Biol. [REDACTED]

Study Completion Date: November 7, 2001



9981036 / MO-01-020753

### Sponsor

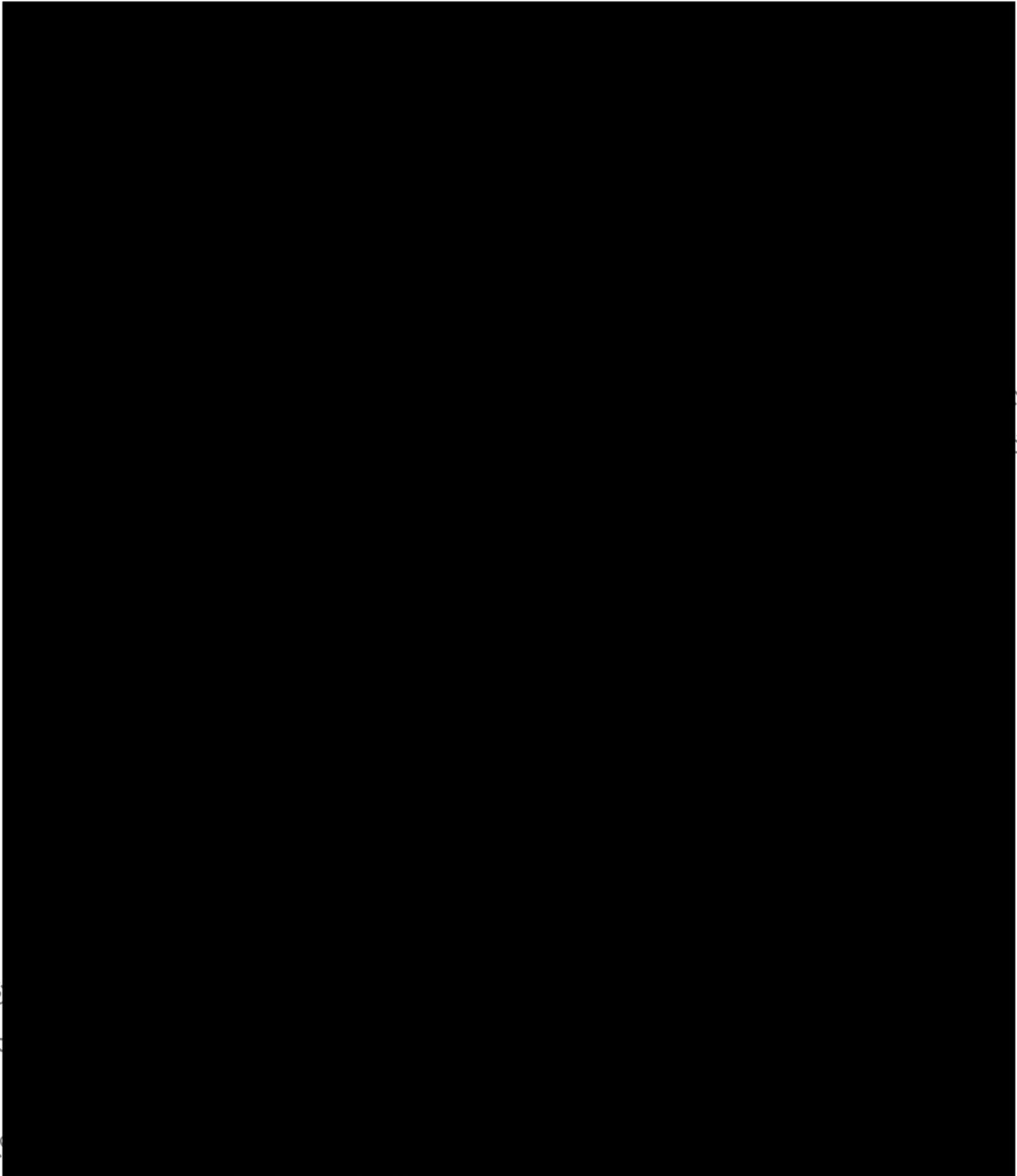
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**Project 9981036**

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## 1. Summary

**Report:** [REDACTED] S. (2001): Effects of Imidacloprid SL 200 (Acute Contact and Oral LD<sub>50</sub>) on Honey Bees (*Apis mellifera* L.) in the Laboratory

**Source:** IBACON, unpublished report No: 9981036, November 7, 2001.

**Guidelines:** OECD 213 and OECD 214

Deviations: None

**GLP:** yes (certified laboratory)

**Material and methods:** Imidacloprid SL 200 (NTN 33893 200 SL), purity: NTN 33893: 194 g/L; (specification: Article No.: 0004958608; Batch No.: 233925888; Tox-No.: 5428-00); under laboratory conditions *Apis mellifera* (30 worker bees per treatment) were exposed for 96 hours to doses of 98.7, 38.5, 11.1, 5.6 and 1.2 ng a.i. per bee for feeding (oral, value based on the actual intake of the test item) and to doses of 800, 400, 200, 100 and 50 ng a.i. per bee for topical application (contact).

The oral and the contact test were prolonged up to 96 hours because of increasing mortality between 24 and 48 hours. The LD<sub>50</sub> of the reference item was 0.19 µg dimethoate per bee in the oral and 0.18 µg Dimethoate per bee in the contact exposure after 24 hours.

**Dates of experimental work:** June 19 to August 3, 2001

**Findings:** Toxicity of Imidacloprid SL 200 to Honey Bees, Laboratory Tests

**Table 1. Summary of mortality of the honey bees in the oral and contact toxicity test.**

| Test item   | Imidacloprid SL 200                                |  |
|---|--|--|
| Test object   | <i>Apis mellifera</i>                              |  |
| Application rates ng a.i./bee   | 98.7*, 38.5*, 11.1*, 5.6* and 1.2*                 | 800, 400, 200, 100 and 50                                    |
| Exposure  | oral<br>(50% sugar solution)                       | contact<br>(solution in water + 1 % wetting agent)           |
| LD <sub>50</sub> ng a.i./bee after 48 h and 96 h (95 % Confidence Limits) | 48 h: 5.6* (3.3 to 9.4)<br>96 h: 5.3* (3.4 to 8.4) | 48 h: 42.2 (20.9 to 85.0)<br>96 h: statistics not applicable |

\* values based on actual intake of the test item

### Observations:

In both, the oral and the contact test the observation period was extended for 48 hours because of delayed mortality.

Behavioural impairments (e.g. apathy or discoordinated movements) were observed for the first 4 hours in the 98.7, 38.5, 11.1 and 5.6 ng a.i./bee dose group. No behavioural impairments were observed in the 1.2 ng a.i. per bee dose group for the whole experimental time. After 24 hours apathy was observed in the 98.7, 38.5 and 11.1 ng a.i. dose group. No further behavioural impairments occurred in the 5.6 ng a.i./bee group. 48 hours following the application three and two bees showed apathy and moving coordination problems in the 98.7 and 38.5 ng a.i. dose group, respectively. After 72 hours two bees were apathetic or showed a discoordinated movement in the group dosed with 98.7 and 38.5 ng a.i./bee. In the 98.7 ng a.i. dose group two bees were found apathetic at the 96 hours check.

In the contact test behavioural impairments of the surviving bees like apathy, vomiting and discoordinated movements occurred in all groups dosed with Imidacloprid SL 200 during the whole experimental time of 96 hours.

3.3 % control mortality was found after 96 hours in the contact test and oral test, respectively.

### Conclusions:

The LD<sub>50</sub> (contact) 48 hrs was determined to be 42.2 ng a.i./bee. The LD<sub>50</sub> (oral) was determined to be 5.6 ng a.i./bee after 48 hrs and 5.3 ng a.i./bee after 96 hrs.

## 2. Survey of the Study

### 2.1 General Information

**Title:** Effects of Imidacloprid SL 200 (Acute Contact and Oral LD<sub>50</sub>) on Honey Bees (*Apis mellifera* L.) in the Laboratory

**Sponsor:** Bayer AG  
Crop Protection Division  
Institute for Environmental Biology  
Alfred-Nobel-Str. 50  
40789 Monheim  
Germany

**Monitoring:** Dr. [REDACTED]

**Test Item:** Imidacloprid SL 200 (NTN 33893 200 SL)

**Test Facility:** Institut für Biologische Analytik  
und Consulting IBACON GmbH  
Arheilger Weg 17  
64380 Rossdorf  
Germany

**IBACON Project:** 9981036

#### Project Staff:

Test Facility Management: Dr. [REDACTED]

Study Director: Dipl. Biol. [REDACTED]

Technical Coordination: [REDACTED]

Head of Quality Assurance Unit (QAU): Dipl. Biol. [REDACTED]

Quality Assurance Unit Manager: Dipl. Biol. [REDACTED]

#### Time Schedule:

Study Initiation Date: April 17, 2001

Receipt of Test Item: November 17, 2000

Experimental Starting Date: June 19, 2001

Experimental Completion Date: August 3, 2001

Draft Report Date: October 22, 2001

Study Completion Date: November 7, 2001

## 2.2 Good Laboratory Practice

This study was performed in compliance with:

- The OECD Principles of Good Laboratory Practice (as revised in 1997) and the
- Chemikaliengesetz ('*Chemicals Act*') der Bundesrepublik Deutschland (ChemG), Anhang I ('*Annex I*'), 1994/97.

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

A quality assurance statement, signed by the Quality Assurance Unit, is included in this final report.

## 2.3 Archiving

The following data / sample(s) will be archived

for 15 years:

- all raw data

- the study protocol

- one certified copy of the final report

for at least 2 years:

- one sample of the test item and of the toxic standard

following the date on which the final report is audited by the Quality Assurance Unit at:

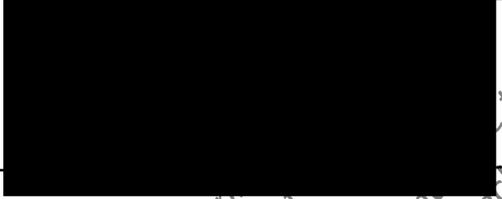
Institut für Biologische Analytik  
und Consulting IBACON GmbH  
Arheilger Weg 17  
64380 Rossdorf  
Germany

No raw data or material relating to the study will be discarded without the sponsor's prior consent.

## 2.4 Signatures

Study Director:

Dipl. Biol. S 



date:

November 7, 2007

Test Facility Management:

Dr. 



date:

November 07, 2007

Sponsor's Representative:

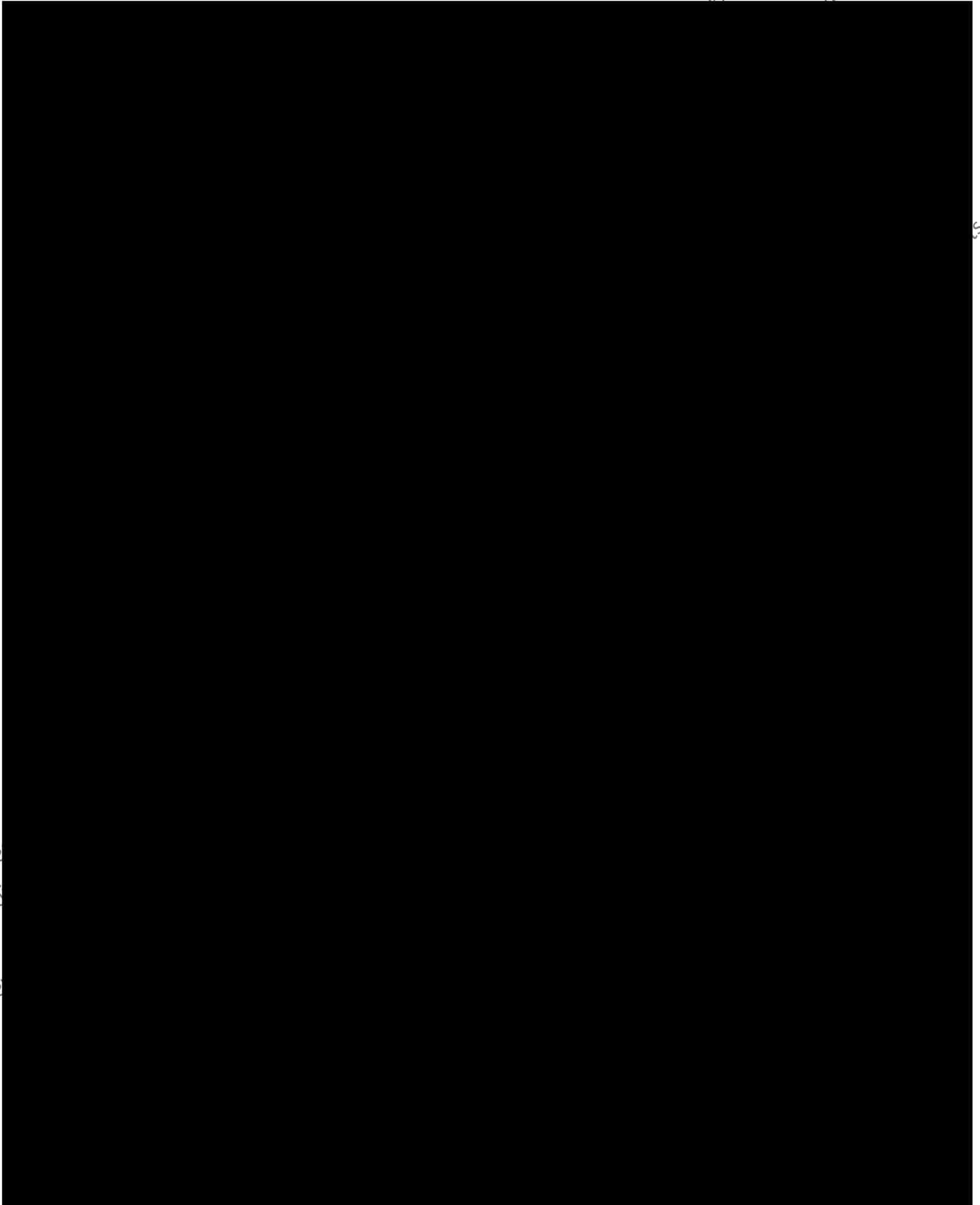
Dr. 



date:

November 13, 2007

### 3. Quality Assurance Unit Statement



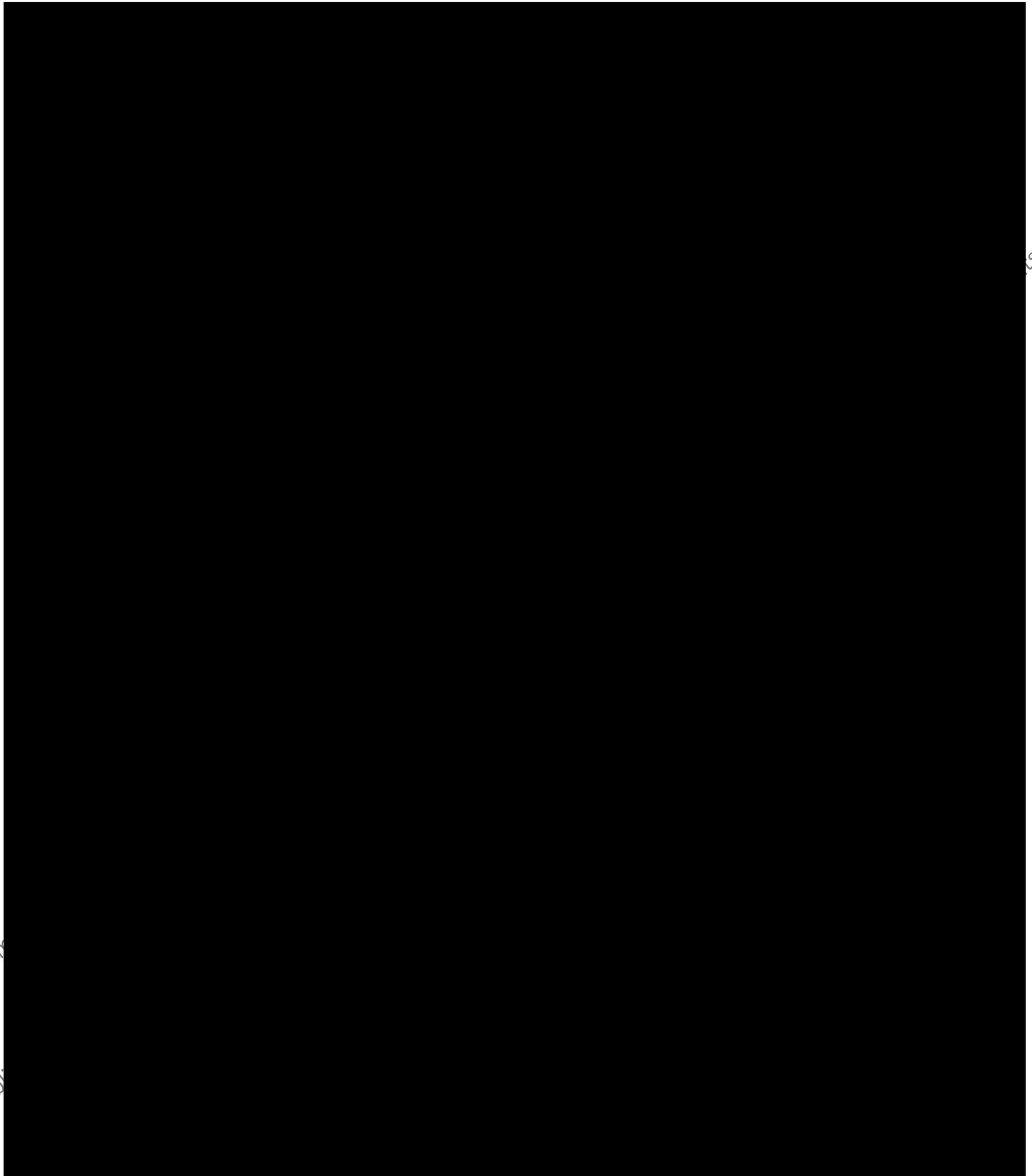
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#### 4. Statement of Compliance



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## 5. Objectives of the Study

### 5.1 Title

Effects of Imidacloprid SL 200 (Acute Contact and Oral LD<sub>50</sub>) on Honey Bees (*Apis mellifera* L.) in the Laboratory

### 5.2 Purpose

Honey bees (*A. mellifera*) can be affected by pesticides-containing residues on plants, via oral intake of contaminated food or water, via inhalation of vapour and directly in the course of the application in the field. If the proposed use pattern of Imidacloprid SL 200 indicates such a possible exposure of honey bees, this study is useful for the registration of the pesticides in question. This study provides:

- the acute toxicity levels of the test item to honey bees;
- toxicity information comparable to expected residues from standard rates, for assessment of the potential hazard to honey bees;
- informational support for precautionary label statements;
- information to indicate the need for further testing or field studies.

### 5.3 Guidelines/Recommendations

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 21<sup>st</sup> September 1998)
- OECD 214: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Contact Toxicity Test, (adopted 21<sup>st</sup> September 1998)
- recent recommendations of the ICPBR group, held in Avignon, France, 1999

### 5.4 Justification for the Selection of the Test System

This test system meets the requirements of the Commission Directive 96/12/EC amending Council Directive 91/414/EEC

## 6. Materials and Methods

### 6.1 Test Item, Control, Wetting Agent and Toxic Standard

#### Test Item

The test item and the information concerning the test item were provided by the sponsor:

Name: Imidacloprid SL 200 (NTN 33893 200 SL)  
Article-No.: 0004958608  
Batch No.: 233925888  
Tox-No.: 5428-00  
Active Ingredient(s)/Purity: NTN 33893: 194 g/L according to certificate of analysis  
CAS No.: 138261-41-3  
Certificate of Analysis Ref. Code / Date: Date of Analysis: September 4, 2000 /  
Date of Certificate: September 20, 2000  
Type: insecticide  
Aggregate State at Room Temperature: liquid  
Colour: light brown  
Density: 1,21 g/cm<sup>3</sup>  
Solubility: in water: miscible  
Stability: pure: see expiry date  
in water: test item is considered stable under test conditions  
Expiry Date: September 4, 2001  
Storage: in original container, at room temperature, in the dark  
Safety Precautions: routine safety and hygienic procedures

#### Control

Oral Test:

tap water

Contact Test:

tap water with 1 % Adhäsit\* with anesthetization

\* (Adhäsit improves spreading of the test droplet on the water repellent hairs on thorax of bees)

## Wetting Agent

### Adhäsit

Name: Adhäsit  
 Batch No.: 0190208  
 Active Ingredient/Purity: 100 g/L Triethanolamin-Dodecylbenzolsulfonat  
 Type: Adhesive  
 Manufacturer: C.F.Spiess & Sohn GmbH & Co.,  
 Hauptstr.4, 67271 Kleinkarlbach  
 Expiry Date: 01/2003  
 Storage: at room temperature, in the dark  
 Target Amount in this Study: 1 %

### Toxic Standard

The information concerning the toxic standard according to the substance container label:

Name: Perfektion EC (BAS 152 110)  
 Manufacturer: BASF AG, Unternehmensbereich Pflanzenschutz, D-67056  
 Ludwigshafen  
 Batch No. (Lot-No.): 99-1  
 Active Ingredient / Purity: Dimethoate: 417.5 g/L analytical (400 g/L nominal),  
 according to certificate of analysis  
 Certificate of Analysis Ref. Code / Date: PCP06027  
 October 16, 2000  
 Type: insecticide  
 Aggregate State at Room Temperature: liquid  
 Colour: blue  
 Density: 1.077 g/cm<sup>3</sup>  
 Solubility: in water: emulsifiable  
 Stability: formulated product: see expiry date  
 in water: toxic standard is considered stable under test  
 conditions  
 Expiry Date: October 2002  
 Storage: in original container, at room temperature, in the dark  
 Amount Applied in this Study: 0.1 - 0.3 µg active ingredient per bee for the contact test  
 0.04 - 0.34 µg active ingredient per bee for the oral test

## 6.2 Test System

Taxonomic Group: worker honey bees (Insecta, Hymenoptera)  
 Species: adult *Apis mellifera* L.  
 Age and Sex: 4 - 6 week old worker bees  
 Origin: honey bee colonies, disease-free and queen-right, bred by IBACON  
 Collection: with glass tubes, from the flight board without anaesthetics, and without the use of smoke, collected in the morning of use

## 6.3 Test Units

Type: stainless steel cages  
 Size: 10 cm x 8.5 cm x 5.5 cm (length x width x height)  
 Front Side: removable glass sheet  
 Bottom: perforated with 98 ventilation holes;  $\phi$  1 mm  
 Inner Walls: lined with filter paper (Co. Macherey & Nagel, D-52355 Düren, Art. No. 68)  
 No. of Individuals: 10 per test unit  
 Replicates: 3 per test item dosages, controls and toxic standard dosages  
 Identification: test units were uniquely identified with study number, application date, treatment, concentration and replicate number

## 6.4 Test Conditions

Test Environment: incubators  
 Temperature: 25 °C  
 Relative Humidity: 40-58%  
 Climatic conditions (daily values) during animal exposure in the incubator

| time   | Temperature [° C] |           | relative Humidity [%] |           |
|--------|-------------------|-----------|-----------------------|-----------|
|        | Contact Test      | Oral Test | Contact Test          | Oral Test |
| 0 hrs  | 25                | 25        | 40                    | 50        |
| 24 hrs | 25                | 25        | 46                    | 44        |
| 48 hrs | 25                | 25        | 53                    | 48        |
| 72 hrs | 25                | 25        | 46                    | 51        |
| 96 hrs | 25                | 25        | 58                    | 50        |

time = time after application; time 0 = application

Light: darkness (except during observation)  
 Ventilation: ventilation to avoid possible accumulation of pesticide vapour  
 Recording: test conditions were recorded with suitable instruments and documented in the raw data

## 6.5 Food

Food: commercial ready-to-use syrup (Apiinvert; 30 % Saccharose, 31 % Glucose, 39 % Fructose) *ad libitum*; was given directly after treatments in syringes; no replacements of the food during the exposure phase

## 6.6 Application of the Test Item, the Control and the Toxic Standard

Application in the Contact Test: one single 5  $\mu$ L droplet of Imidacloprid SL 200 in solvent (solvent = water + 1 % Adhäsit\*) was placed on the dorsal bee thorax using a Burkard + Applicator.

For the controls one 5  $\mu$ L droplet of tap water with 1 % Adhäsit was used (a single 5  $\mu$ L droplet was chosen in deviation to the guideline recommendation of 1  $\mu$ L) since a higher volume ensured a more reliable dispersion of the test items; Ibacon experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are expected).

Application in the Oral Test: *ca.* 20 mg food (*ca.* 20  $\mu$ L ready-to-use syrup) per bee was mixed with Imidacloprid SL 200, toxic standard and tap water (test item solution and sugar were mixed together in a way that the final sugar solution was 50%). this diet was offered in syringes which were weighed before and after introduction into the cages (duration of uptake did not exceed 6 hours)

Dosages of the Test Item: the dosages applied were adjusted to reflect the percentage a.i.

Nominal Dosage of the Test Item in the Contact Test: 800, 400, 200, 100 and 50 ng of the a.i. of Imidacloprid SL 200/bee

Measured Dosage of the Test Item in the Oral Test: 98.7, 38.9, 13.1, 5.6 and 1.2 ng of the a.i. of Imidacloprid SL 200/bee

Dosages of the Toxic Standard: 0.30, 0.20, 0.15, and 0.10  $\mu$ g Dimethoate per bee (contact test)  
0.34, 0.17, 0.09 and 0.04  $\mu$ g Dimethoate per bee (oral test)

Controls: contact test: CO<sub>2</sub>/tap water+Adhäsit\* treated control  
oral test: tap water control

\*The Adhäsit was used to improve the spreading of the test droplet on the bee body. Adhäsit is non-toxic to honey bees.

## 6.7 Course of the Test

Replicates: 3 per dose level/controls

Individuals: 10 per unit, 30 individuals per treatment group

Anaesthetization: bees were anaesthetized with CO<sub>2</sub> (only in the contact test)

Starvation Time: 65 minutes (only in the oral test)

Exposure Time: 96 hours

Prolongation: the contact and oral test was prolonged up to 96 hours (because of increasing mortality between 24 and 48 hours)

## 6.8 Test Parameters

|                            |   |
|----------------------------|---|
| Mortality:                 | number of dead bees after 4 hours (first day) and 24, 48, 72 and 96 hours |
| Behavioural Abnormalities: | number of dead bees after 4 hours (first day) and 24, 48, 72 and 96 hours |

## 6.9 Result Evaluation

|            |  |
|------------|--|
| Mortality: | results obtained from the bees treated with test item are compared to those obtained from the toxic standard and the controls.<br>The contact and oral LD <sub>50</sub> of the test item and the toxic standard were estimated with Probit Analysis (according to Finney (1971) and Moving Average computations (after Thompson (1947)). The computer program used to perform the statistical analyses was EASY ASSAY Critical Values (Ratte, 1997) Version 3.0. |
|------------|--|

## 6.10 Validity Criteria of the Study

|                                     |   |
|-------------------------------------|---|
| Control Mortality:                  | must not exceed 10 % (see 7.1)  |
| LD <sub>50</sub> of Toxic Standard: | contact test: must be in the range of 0.10 - 0.30 µg a.i./bee (see 7.1)<br>oral test: must be in the range of 0.10 - 0.35 µg a.i./bee (see 7.1) |

## 6.11 Deviations to the Study Protocol

no deviations to the study protocol occurred

## 7. Results and Discussion

### 7.1 Validity Criteria of the Study

Control Mortality:

Contact Test  
water Control: 3.3 %

Oral Test  
water Control: 3.3 %

LD<sub>50</sub> of Toxic Standard (24 hrs):

Contact Test 0.18 µg a.i./bee

Oral Test 0.19 µg a.i./bee

### 7.2 Contact Toxicity Test

Mortality:

mortality occurred in all treatment groups in a dose related pattern.

Control mortality was 3.3 %

Prolongation:

was necessary because of delayed mortality between 24 and 48 hours

Behavioural Abnormalities:

behavioural abnormalities attributed to exposure of the test organisms to the test item were vomiting, apathy and discoordinated movements

Test Item Contact LD<sub>50</sub>:

|                                 | 24 h             | 48 h             | 72 h             | 96 h |
|---------------------------------|------------------|------------------|------------------|------|
|                                 | 60.8 ng a.i./bee | 42.2 ng a.i./bee | 17.3 ng a.i./bee | n.d. |
| 95 %- Confidence limit (lower): | 37.2 ng a.i./bee | 20.9 ng a.i./bee | 3.7 ng a.i./bee  | n.d. |
| 95 %- Confidence limit (upper): | 99.3 ng a.i./bee | 85.0 ng a.i./bee | 80.4 ng a.i./bee | n.d. |

n.d. = not detectable, due to a too low number of survivors

Toxic Standard Contact LD<sub>50</sub>:

|                                 | 24 h             | 48 h             | 72 h             | 96 h             |
|---------------------------------|------------------|------------------|------------------|------------------|
|                                 | 0.18 µg a.i./bee | 0.15 µg a.i./bee | 0.14 µg a.i./bee | 0.13 µg a.i./bee |
| 95 %- Confidence limit (lower): | 0.16 µg a.i./bee | 0.14 µg a.i./bee | 0.12 µg a.i./bee | 0.12 µg a.i./bee |
| 95 %- Confidence limit (upper): | 0.19 µg a.i./bee | 0.16 µg a.i./bee | 0.15 µg a.i./bee | 0.15 µg a.i./bee |

Tables:

see Table 2; for details see Appendix Table 4 and 5

**Table 2. Mortality<sup>a</sup> and behavioural abnormalities<sup>a</sup> of the bees in the contact toxicity test<sup>b</sup>**

| test item          | after 24 hours |                | after 48 hours |                | after 72 hours |                | after 96 hours |                |
|--------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                    | mortality      | behav. abnorm. |
|                    | mean           |
| dosage ng a.i./bee | %              | %              | %              | %              | %              | %              | %              | %              |
| 800                | 90.0           | 10.0           | 90.0           | 10.0           | 93.3           | 3.3            | 100.0          | 0.0            |
| 400                | 100.0          | 0.0            | 100.0          | 0.0            | 100.0          | 0.0            | 100.0          | 0.0            |
| 200                | 93.3           | 6.7            | 96.7           | 3.3            | 100.0          | 0.0            | 100.0          | 0.0            |
| 100                | 63.3           | 36.7           | 76.7           | 13.3           | 90.0           | 10.0           | 96.7           | 3.3            |
| 50                 | 36.7           | 60.0           | 43.3           | 43.3           | 60.0           | 26.7           | 80.0           | 20.0           |
| water              | 0.0            | 3.3            | 3.3            | 0.0            | 3.3            | 0.0            | 3.3            | 0.0            |
| Toxic Standard     |                |                |                |                |                |                |                |                |
| [µg a.i.]          |                |                |                |                |                |                |                |                |
| 0.30               | 93.3           | 6.7            | 100.0          | 0.0            | 100.0          | 0.0            | 100.0          | 0.0            |
| 0.20               | 76.7           | 23.3           | 90.0           | 6.7            | 90.0           | 10.0           | 96.7           | 0.0            |
| 0.15               | 23.3           | 43.3           | 46.7           | 26.7           | 60.0           | 0.0            | 63.3           | 3.3            |
| 0.10               | 3.3            | 6.7            | 10.0           | 3.3            | 20.0           | 0.0            | 20.0           | 0.0            |

<sup>a</sup> results are averages from three replicates (ten bees each) per dosage/control

<sup>b</sup> see Appendix, Table 4 for details;

behav. abnorm. = behavioural abnormalities; water = CO<sub>2</sub>/water-treated control

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### 7.3 Oral Toxicity Test

Mortality: mortality occurred in all treatment groups in a dose related pattern.  
Control mortality was 3.3 %

Prolongation: was necessary because of delayed mortality between 24 and 48 hours

Behavioural Abnormalities: behavioural abnormalities attributed to exposure of the test organisms to the test item were disorganised movements and apathy

Test Item Oral LD<sub>50</sub>:

|                                 | 24 h             | 48 h            | 72 h            | 96 h            |
|---------------------------------|------------------|-----------------|-----------------|-----------------|
|                                 | 6.9 ng a.i./bee  | 5.6 ng a.i./bee | 5.4 ng a.i./bee | 3.3 ng a.i./bee |
| 95 %- Confidence limit (lower): | 3.3 ng a.i./bee  | 3.3 ng a.i./bee | 3.3 ng a.i./bee | 3.4 ng a.i./bee |
| 95 %- Confidence limit (upper): | 14.7 ng a.i./bee | 9.4 ng a.i./bee | 8.7 ng a.i./bee | 8.4 ng a.i./bee |

Toxic Standard Oral LD<sub>50</sub>:

|                                 | 24 h             | 48 h             | 72 h             | 96 h             |
|---------------------------------|------------------|------------------|------------------|------------------|
|                                 | 0.19 µg a.i./bee | 0.14 µg a.i./bee | 0.13 µg a.i./bee | 0.12 µg a.i./bee |
| 95 %- Confidence limit (lower): | 0.16 µg a.i./bee | 0.12 µg a.i./bee | 0.11 µg a.i./bee | 0.10 µg a.i./bee |
| 95 %- Confidence limit (upper): | 0.23 µg a.i./bee | 0.16 µg a.i./bee | 0.15 µg a.i./bee | 0.14 µg a.i./bee |

Tables:

see Table 3; for details see Appendix Table 6 and 7

**Table 3. Mortality<sup>a</sup> and behavioural abnormalities<sup>b</sup> of the bees in the oral toxicity test<sup>b</sup>**

| uptaken test item<br>ng a.i./bee | after 24 hours         |                             | after 48 hours         |                             | after 72 hours         |                             | after 96 hours         |                             |
|----------------------------------|------------------------|-----------------------------|------------------------|-----------------------------|------------------------|-----------------------------|------------------------|-----------------------------|
|                                  | mortality<br>mean<br>% | behav. abnorm.<br>mean<br>% |
| 98.7                             | 43.3                   | 26.7                        | 73.3                   | 10.0                        | 90.0                   | 6.7                         | 93.3                   | 6.7                         |
| 38.5                             | 56.7                   | 36.7                        | 76.7                   | 6.7                         | 83.3                   | 6.7                         | 86.7                   | 0.0                         |
| 11.1                             | 63.3                   | 10.0                        | 66.7                   | 0.0                         | 66.7                   | 0.0                         | 66.7                   | 0.0                         |
| 5.6                              | 53.3                   | 0.0                         | 53.3                   | 0.0                         | 53.3                   | 0.0                         | 53.3                   | 0.0                         |
| 1.2                              | 0.0                    | 0.0                         | 0.0                    | 0.0                         | 0.0                    | 0.0                         | 0.0                    | 0.0                         |
| water                            | 0.0                    | 0.0                         | 0.0                    | 0.0                         | 3.3                    | 0.0                         | 3.3                    | 0.0                         |
| Toxic Standard [µg a.i.]         |                        |                             |                        |                             |                        |                             |                        |                             |
| 0.34                             | 83.3                   | 0.0                         | 96.7                   | 3.3                         | 96.7                   | 3.3                         | 96.7                   | 0.0                         |
| 0.17                             | 43.3                   | 13.3                        | 66.7                   | 0.0                         | 70.0                   | 6.7                         | 73.3                   | 0.0                         |
| 0.09                             | 6.7                    | 3.3                         | 16.7                   | 0.0                         | 16.7                   | 0.0                         | 16.7                   | 0.0                         |
| 0.04                             | 0.0                    | 3.3                         | 3.3                    | 0.0                         | 6.7                    | 0.0                         | 13.3                   | 0.0                         |

<sup>a</sup> results are averages from three replicates (ten bees each) per dosage/control

<sup>b</sup> see Appendix, Table 6 for details

behav. abnorm. = behavioural abnormalities

## 7.4 Conclusions

Mortality:

- Contact LD<sub>50</sub> (24h) of Imidacloprid SL 200: 60.8 ng a.i./bee
- Contact LD<sub>50</sub> (48h) of Imidacloprid SL 200: 42.2 ng a.i./bee
- Contact LD<sub>50</sub> (72h) of Imidacloprid SL 200: 17.3 ng a.i./bee
- Contact LD<sub>50</sub> (96h) of Imidacloprid SL 200: not detectable
- Oral LD<sub>50</sub> (24h) of Imidacloprid SL 200: 6.9 ng a.i./bee
- Oral LD<sub>50</sub> (48h) of Imidacloprid SL 200: 5.6 ng a.i./bee
- Oral LD<sub>50</sub> (72h) of Imidacloprid SL 200: 5.4 ng a.i./bee
- Oral LD<sub>50</sub> (96h) of Imidacloprid SL 200: 5.3 ng a.i./bee

Behavioural Abnormalities: behavioural abnormalities attributed to exposure of the test organisms to the test item were e.g. discoordinated movements, vomiting and apathy during the experimental time

## 8. References

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## 9. Distribution of the Final Report

Sponsor: 1x (the original final report )

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**Table 4. (Exact Data). Definitive contact toxicity test: mortality and behavioural abnormalities of the bees**

| test item | dosage ng a.i./bee | after 4 hours |               |       | after 24 hours |               |       | after 48 hours |               |       | after 72 hours |               |       | after 96 hours |               |       |
|-----------|--------------------|---------------|---------------|-------|----------------|---------------|-------|----------------|---------------|-------|----------------|---------------|-------|----------------|---------------|-------|
|           |                    | dead #        | beh. abnor. # | symp. | dead #         | beh. abnor. # | symp. | dead #         | beh. abnor. # | symp. | dead #         | beh. abnor. # | symp. | dead #         | beh. abnor. # | symp. |
| 1         | 800                | 8             | 2             | a,c   | 9              | 1             | c     | 9              | 1             | c     | 9              | 1             | c     | 10             | 0             |       |
| 2         | 800                | 10            | 0             |       | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       |
| 3         | 800                | 8             | 2             | a,c   | 8              | 2             | c     | 8              | 2             | c     | 9              | 0             |       | 10             | 0             |       |
| 1         | 400                | 10            | 0             |       | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       |
| 2         | 400                | 9             | 1             | a     | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       |
| 3         | 400                | 9             | 1             | a     | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       |
| 1         | 200                | 9             | 1             | c     | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       |
| 2         | 200                | 9             | 1             | a     | 9              | 1             | a     | 9              | 1             | a     | 10             | 0             |       | 10             | 0             |       |
| 3         | 200                | 9             | 1             | a     | 9              | 1             | a     | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       |
| 1         | 100                | 5             | 5             | c     | 9              | 1             | c     | 9              | 1             | c     | 9              | 1             | c     | 9              | 1             | c     |
| 2         | 100                | 5             | 5             | c     | 5              | 5             | c     | 7              | 5             | c     | 8              | 2             | c     | 10             | 0             |       |
| 3         | 100                | 5             | 5             | c     | 5              | 5             | c     | 7              | 5             | c     | 10             | 0             |       | 10             | 0             |       |
| 1         | 50                 | 3             | 7             | a,c   | 5              | 5             | c     | 5              | 5             | c     | 7              | 2             | b     | 9              | 1             | c     |
| 2         | 50                 | 8             | 2             | a,c   | 5              | 5             | c     | 5              | 5             | c     | 7              | 2             | b     | 7              | 3             | b,c   |
| 3         | 50                 | 0             | 10            | a,c   | 1              | 8             | c     | 3              | 5             | b,c   | 4              | 4             | a     | 8              | 2             | a,c   |
| 1         | control            | 0             | 0             |       | 0              | 0             |       | 0              | 0             |       | 0              | 0             |       | 0              | 0             |       |
| 2         | water control      | 0             | 0             |       | 0              | 0             |       | 0              | 0             |       | 0              | 0             |       | 0              | 0             |       |
| 3         | control            | 0             | 0             |       | 0              | 0             |       | 0              | 0             |       | 0              | 0             |       | 0              | 0             |       |
| 1         | toxic              | 0.30          | 3             | 4     | b              | 10            | 0     | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       |
| 2         | standard           | 0.30          | 3             | 4     | b              | 10            | 0     | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       |
| 3         | [µg a.i.]          | 0.30          | 4             | 3     | b              | 8             | 2     | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       |
| 1         | toxic              | 0.20          | 3             | 2     | c              | 10            | 0     | 10             | 0             |       | 10             | 0             |       | 10             | 0             |       |
| 2         | standard           | 0.20          | 0             | 3     | b              | 8             | 2     | 9              | 1             | b     | 9              | 1             | b     | 10             | 0             |       |
| 3         | [µg a.i.]          | 0.20          | 0             | 0     | 5              | 5             | b     | 8              | 1             | b     | 9              | 2             | b     | 9              | 0             |       |
| 1         | toxic              | 0.15          | 0             | 0     | 4              | 4             | b     | 4              | 4             | b     | 5              | 0             |       | 4              | 0             |       |
| 2         | standard           | 0.15          | 0             | 0     | 2              | 4             | b     | 3              | 2             | b     | 4              | 0             |       | 6              | 1             | b     |
| 3         | [µg a.i.]          | 0.15          | 0             | 0     | 4              | 5             | b,c   | 7              | 2             | b     | 0              | 0             |       | 9              | 0             |       |
| 1         | toxic              | 0.10          | 0             | 0     | 1              | 1             | b     | 0              | 0             |       | 3              | 0             |       | 3              | 0             |       |
| 2         | standard           | 0.10          | 0             | 0     | 1              | 1             | b     | 3              | 0             |       | 0              | 0             |       | 3              | 0             |       |
| 3         | [µg a.i.]          | 0.10          | 0             | 0     | 0              | 0             |       | 0              | 0             |       | 0              | 0             |       | 0              | 0             |       |

beh. abnor. behavioural abnormalities; # number; symp. = observed symptoms according to the following key:  
a = food refusal/vomiting; b = moving coordination problems; c = apathy; d = intensive cleaning; e = nervous

**Table 5. (Relative Data). Definitive contact toxicity test: mortality and behavioural abnormalities of the bees**

| test item          | after 24 hours |       |                         |       | after 48 hours |       |                         |       | after 72 hours |       |                         |       | after 96 hours |       |                         |       |
|--------------------|----------------|-------|-------------------------|-------|----------------|-------|-------------------------|-------|----------------|-------|-------------------------|-------|----------------|-------|-------------------------|-------|
|                    | mortality      |       | beh.abnor. <sup>a</sup> |       | mortality      |       | beh.abnor. <sup>a</sup> |       | mortality      |       | beh.abnor. <sup>a</sup> |       | mortality      |       | beh.abnor. <sup>a</sup> |       |
| dosage ng a.i./bee | mean %         | ±SD % | mean %                  | ±SD % | mean %         | ±SD % | mean %                  | ±SD % | mean %         | ±SD % | mean %                  | ±SD % | mean %         | ±SD % | mean %                  | ±SD % |
| 800                | 90.0           | 10.0  | 10.0                    | 10.0  | 90.0           | 10.0  | 10.0                    | 10.0  | 93.3           | 5.8   | 3.3                     | 5.8   | 100.0          | 0.0   | 0.0                     | 0.0   |
| 400                | 100.0          | 0.0   | 0.0                     | 0.0   | 100.0          | 0.0   | 0.0                     | 0.0   | 100.0          | 0.0   | 0.0                     | 0.0   | 100.0          | 0.0   | 0.0                     | 0.0   |
| 200                | 93.3           | 5.8   | 6.7                     | 5.8   | 96.7           | 5.8   | 9.3                     | 5.8   | 100.0          | 0.0   | 0.0                     | 0.0   | 100.0          | 0.0   | 0.0                     | 0.0   |
| 100                | 63.3           | 23.1  | 36.7                    | 23.1  | 76.7           | 11.5  | 13.3                    | 5.8   | 90.0           | 10.0  | 10.0                    | 10.0  | 96.7           | 5.8   | 3.3                     | 5.8   |
| 50                 | 36.7           | 23.1  | 60.0                    | 17.3  | 43.3           | 11.5  | 43.3                    | 11.5  | 60.0           | 17.3  | 26.7                    | 11.5  | 80.0           | 10.0  | 20.0                    | 10.0  |
| water              | 0.0            | 0.0   | 3.3                     | 5.8   | 3.3            | 5.8   | 0.0                     | 0.0   | 3.3            | 5.8   | 0.0                     | 0.0   | 3.3            | 5.8   | 0.0                     | 0.0   |
| Toxic Standard     |                |       |                         |       |                |       |                         |       |                |       |                         |       |                |       |                         |       |
| 0.30               | 93.3           | 11.5  | 6.7                     | 11.5  | 100.0          | 0.0   | 0.0                     | 0.0   | 100.0          | 0.0   | 0.0                     | 0.0   | 100.0          | 0.0   | 0.0                     | 0.0   |
| 0.20               | 76.7           | 25.2  | 23.3                    | 25.2  | 90.0           | 10.0  | 6.7                     | 5.8   | 90.0           | 10.0  | 10.0                    | 10.0  | 96.7           | 5.8   | 0.0                     | 0.0   |
| 0.15               | 23.3           | 15.3  | 43.3                    | 5.8   | 46.7           | 20.8  | 26.7                    | 11.5  | 60.0           | 26.5  | 0.0                     | 0.0   | 63.3           | 25.2  | 3.3                     | 5.8   |
| 0.10               | 3.3            | 5.8   | 6.7                     | 5.8   | 10.0           | 17.3  | 3.3                     | 5.8   | 20.0           | 17.3  | 0.0                     | 0.0   | 20.0           | 17.3  | 0.0                     | 0.0   |

<sup>a</sup>beh. abnor. behavioural abnormalities; mean = mean of three replicates;  
±SD = standard deviation from three replications  
water = CO<sub>2</sub>/water-treated control

**Table 6. (Exact Data). Definitive oral toxicity test: mortality and behavioural abnormalities of the bees**

| unit no.: | test item dosage ng a.i./bee | after 4 hours |                     | after 24 hours |                     | after 48 hours |                     | after 72 hours |                     | after 96 hours |                     |
|-----------|------------------------------|---------------|---------------------|----------------|---------------------|----------------|---------------------|----------------|---------------------|----------------|---------------------|
|           |                              | dead #        | beh. abnor. # symp. | dead #         | beh. abnor. # symp. | dead #         | beh. abnor. # symp. | dead #         | beh. abnor. # symp. | dead #         | beh. abnor. # symp. |
| 1         | 90.5                         | 0             | 10 c                | 7              | 3 c                 | 10             | 0                   | 10             | 0                   | 10             | 0                   |
| 2         | 91.0                         | 0             | 10 c                | 4              | 6 c                 | 7              | 0                   | 10             | 0                   | 10             | 0                   |
| 3         | 114.5                        | 0             | 10 c                | 2              | 8 c                 | 5              | 3 b,c               | 7              | 2 b,c               | 8              | 2 c                 |
| 1         | 30.8                         | 0             | 10 c                | 7              | 3 c                 | 10             | 0                   | 10             | 0                   | 10             | 0                   |
| 2         | 38.3                         | 0             | 10 c                | 5              | 4 c                 | 6              | 1                   | 7              | 0                   | 7              | 0                   |
| 3         | 46.5                         | 0             | 10 c                | 5              | 4 c                 | 7              | 1                   | 8              | 0                   | 9              | 0                   |
| 1         | 11.3                         | 1             | 9 b,c               | 6              | 0                   | 6              | 0                   | 6              | 0                   | 6              | 0                   |
| 2         | 11.2                         | 0             | 10 b,c              | 6              | 0                   | 6              | 0                   | 6              | 0                   | 6              | 0                   |
| 3         | 10.9                         | 0             | 10 b,c              | 7              | 3 c                 | 8              | 0                   | 8              | 0                   | 8              | 0                   |
| 1         | 5.8                          | 0             | 6 b,c               | 3              | 0                   | 3              | 0                   | 3              | 0                   | 3              | 0                   |
| 2         | 5.8                          | 0             | 10 b,c              | 6              | 0                   | 6              | 0                   | 6              | 0                   | 6              | 0                   |
| 3         | 5.3                          | 0             | 10 b,c              | 7              | 0                   | 7              | 0                   | 7              | 0                   | 7              | 0                   |
| 1         | 1.2                          | 0             | 0                   | 0              | 0                   | 0              | 0                   | 0              | 0                   | 0              | 0                   |
| 2         | 1.1                          | 0             | 0                   | 0              | 0                   | 0              | 0                   | 0              | 0                   | 0              | 0                   |
| 3         | 1.2                          | 0             | 0                   | 0              | 0                   | 0              | 0                   | 0              | 0                   | 0              | 0                   |
| 1         | control                      | 0             | 0                   | 0              | 0                   | 0              | 0                   | 0              | 0                   | 0              | 0                   |
| 2         | water control                | 0             | 0                   | 0              | 0                   | 0              | 0                   | 0              | 0                   | 0              | 0                   |
| 3         | control                      | 0             | 0                   | 0              | 0                   | 0              | 0                   | 1              | 0                   | 1              | 0                   |
| 1         | toxic 0.33                   | 1             | 9 b,c               | 10             | 0                   | 10             | 0                   | 10             | 0                   | 10             | 0                   |
| 2         | standard 0.35                | 1             | 9 b,c               | 7              | 0                   | 7              | 0                   | 10             | 0                   | 10             | 0                   |
| 3         | [µg a.i.] 0.34               | 0             | 8 b,c               | 8              | 0                   | 9              | 1 c                 | 9              | 0                   | 9              | 0                   |
| 1         | toxic 0.16                   | 0             | 4 b                 | 5              | 0                   | 5              | 0                   | 8              | 0                   | 8              | 0                   |
| 2         | standard 0.17                | 0             | 4 b                 | 5              | 2 b,c               | 7              | 0                   | 8              | 2 b,c               | 9              | 0                   |
| 3         | [µg a.i.] 0.17               | 2             | 3 b                 | 5              | 1 b                 | 8              | 0                   | 8              | 0                   | 8              | 0                   |
| 1         | toxic 0.09                   | 0             | 1 e                 | 0              | 0                   | 1              | 0                   | 2              | 0                   | 1              | 0                   |
| 2         | standard 0.08                | 0             | 1 e                 | 1              | 1 c                 | 2              | 0                   | 2              | 0                   | 2              | 0                   |
| 3         | [µg a.i.] 0.09               | 0             | 0 e                 | 0              | 0                   | 2              | 0                   | 0              | 0                   | 2              | 0                   |
| 1         | toxic 0.04                   | 0             | 0                   | 0              | 0                   | 0              | 0                   | 1              | 0                   | 3              | 0                   |
| 2         | standard 0.04                | 0             | 0                   | 0              | 1 c                 | 1              | 0                   | 0              | 0                   | 1              | 0                   |
| 3         | [µg a.i.] 0.04               | 0             | 0                   | 0              | 0                   | 0              | 0                   | 0              | 0                   | 0              | 0                   |

beh. abnor. = behavioural abnormalities; # = number; symp. = observed symptoms according to the following key:  
a = food refusal/vomiting; b = moving coordination problems; c = apathy; d = intensive cleaning; e = nervous

**Table 7. (Relative Data). Definitive oral toxicity test: mortality and behavioural abnormalities of the bees**

| test item dosage ng a.i./bee | after 24 hours |       |                         |       | after 48 hours |       |                         |       | after 72 hours |       |                         |       | after 96 hours |       |                         |       |
|------------------------------|----------------|-------|-------------------------|-------|----------------|-------|-------------------------|-------|----------------|-------|-------------------------|-------|----------------|-------|-------------------------|-------|
|                              | mortality      |       | beh.abnor. <sup>a</sup> |       | mortality      |       | beh.abnor. <sup>a</sup> |       | mortality      |       | beh.abnor. <sup>a</sup> |       | mortality      |       | beh.abnor. <sup>a</sup> |       |
|                              | mean %         | ±SD % | mean %                  | ±SD % | mean %         | ±SD % | mean %                  | ±SD % | mean %         | ±SD % | mean %                  | ±SD % | mean %         | ±SD % | mean %                  | ±SD % |
| 98.8                         | 43.3           | 25.2  | 56.7                    | 25.2  | 73.3           | 35.2  | 10.0                    | 10.3  | 00.0           | 17.3  | 6.7                     | 11.5  | 93.3           | 11.5  | 6.7                     | 11.5  |
| 98.5                         | 56.7           | 14.5  | 36.7                    | 5.8   | 76.7           | 20.8  | 0.0                     | 5.8   | 83.3           | 15.3  | 6.7                     | 11.5  | 86.7           | 15.3  | 0.0                     | 0.0   |
| 114.5                        | 60.3           | 5.8   | 10.0                    | 17.3  | 66.7           | 11.5  | 0.0                     | 0.0   | 66.7           | 11.5  | 0.0                     | 0.0   | 66.7           | 11.5  | 0.0                     | 0.0   |
| 30.8                         | 53.3           | 20.8  | 0.0                     | 0.0   | 53.3           | 20.8  | 0.0                     | 0.0   | 53.3           | 20.8  | 0.0                     | 0.0   | 53.3           | 20.8  | 0.0                     | 0.0   |
| 11.3                         | 0.0            | 0.0   | 0.0                     | 0.0   | 0.0            | 0.0   | 0.0                     | 0.0   | 0.0            | 0.0   | 0.0                     | 0.0   | 0.0            | 0.0   | 0.0                     | 0.0   |
| 11.2                         | 0.0            | 0.0   | 0.0                     | 0.0   | 0.0            | 0.0   | 0.0                     | 0.0   | 0.0            | 0.0   | 0.0                     | 0.0   | 0.0            | 0.0   | 0.0                     | 0.0   |
| 10.9                         | 0.0            | 0.0   | 0.0                     | 0.0   | 0.0            | 0.0   | 0.0                     | 0.0   | 3.3            | 5.8   | 0.0                     | 0.0   | 3.3            | 5.8   | 0.0                     | 0.0   |
| control                      | 0.0            | 0.0   | 0.0                     | 0.0   | 0.0            | 0.0   | 0.0                     | 0.0   |                |       |                         |       |                |       |                         |       |
| water control                |                |       |                         |       |                |       |                         |       |                |       |                         |       |                |       |                         |       |
| control                      |                |       |                         |       |                |       |                         |       |                |       |                         |       |                |       |                         |       |
| toxic 0.33                   | 83.3           | 15.3  | 0.0                     | 0.0   | 96.7           | 5.8   | 3.3                     | 5.8   | 96.7           | 5.8   | 3.3                     | 5.8   | 96.7           | 5.8   | 0.0                     | 0.0   |
| standard 0.35                | 43.3           | 11.5  | 15.3                    | 11.5  | 66.7           | 15.3  | 0.0                     | 0.0   | 70.0           | 17.3  | 6.7                     | 11.5  | 73.3           | 20.8  | 0.0                     | 0.0   |
| [µg a.i.] 0.34               | 6.7            | 5.8   | 3.3                     | 5.8   | 16.7           | 5.8   | 0.0                     | 0.0   | 16.7           | 5.8   | 0.0                     | 0.0   | 16.7           | 5.8   | 0.0                     | 0.0   |
| toxic 0.16                   | 0.0            | 0.0   | 0.0                     | 0.0   | 0.0            | 0.0   | 0.0                     | 0.0   | 6.7            | 5.8   | 0.0                     | 0.0   | 13.3           | 15.3  | 0.0                     | 0.0   |
| standard 0.17                |                |       |                         |       |                |       |                         |       |                |       |                         |       |                |       |                         |       |
| [µg a.i.] 0.17               |                |       |                         |       |                |       |                         |       |                |       |                         |       |                |       |                         |       |
| toxic 0.09                   |                |       |                         |       |                |       |                         |       |                |       |                         |       |                |       |                         |       |
| standard 0.08                |                |       |                         |       |                |       |                         |       |                |       |                         |       |                |       |                         |       |
| [µg a.i.] 0.09               |                |       |                         |       |                |       |                         |       |                |       |                         |       |                |       |                         |       |
| toxic 0.04                   |                |       |                         |       |                |       |                         |       |                |       |                         |       |                |       |                         |       |
| standard 0.04                |                |       |                         |       |                |       |                         |       |                |       |                         |       |                |       |                         |       |
| [µg a.i.] 0.04               |                |       |                         |       |                |       |                         |       |                |       |                         |       |                |       |                         |       |

<sup>a</sup>beh.abnor. = behavioural abnormalities; mean = mean of three replicates;  
±SD = standard deviation from three replications;  
water = water-treated control

**Table 8.** Definitive oral intake

| test item dosage<br>nominal         | test item<br>concentrations<br>in food<br>ng a.i./ mg | test item<br># | weight of syringes       |                        |                  | uptaken<br>solution |                           | uptaken<br>test item      |  |
|-------------------------------------|---|----------------|--------------------------|------------------------|------------------|---------------------|---------------------------|---------------------------|--|
|                                     |   |                | start <sup>a</sup><br>mg | end <sup>b</sup><br>mg | difference<br>mg | mg/bee              | ng a.i./ bee <sup>d</sup> | ng a.i./ bee <sup>c</sup> |  |
| 100 ng a.i./bee                     | 5   | 1              | 11245                    | 11064                  | 181              | 18                  | 90.5                      |                           |  |
|                                     |   | 2              | 11170                    | 10988                  | 182              | 18                  | 91.0                      | 98.7                      |  |
|                                     |   | 3              | 10488                    | 10259                  | 229              | 23                  | 114.5                     |                           |  |
| 50 ng a.i./bee                      | 2.5   | 1              | 11195                    | 11072                  | 123              | 12                  | 30.8                      |                           |  |
|                                     |   | 2              | 10516                    | 10363                  | 153              | 15                  | 38.3                      | 38.5                      |  |
|                                     |   | 3              | 11247                    | 11061                  | 186              | 19                  | 46.5                      |                           |  |
| 10 ng a.i./ bee                     | 0.5   | 1              | 11133                    | 10907                  | 226              | 23                  | 11.3                      |                           |  |
|                                     |   | 2              | 11165                    | 10942                  | 223              | 22                  | 11.2                      | 11.1                      |  |
|                                     |   | 3              | 10624                    | 10406                  | 218              | 22                  | 10.9                      |                           |  |
| 5 ng a.i./ bee                      | 0.25  | 1              | 10576                    | 10344                  | 232              | 23                  | 5.8                       |                           |  |
|                                     |   | 2              | 11144                    | 10912                  | 232              | 23                  | 5.8                       | 5.6                       |  |
|                                     |   | 3              | 11221                    | 10910                  | 211              | 21                  | 5.3                       |                           |  |
| 1 ng a.i./ bee                      | 0.05  | 1              | 11139                    | 10904                  | 235              | 24                  | 1.2                       |                           |  |
|                                     |   | 2              | 11264                    | 11038                  | 226              | 23                  | 1.1                       | 1.2                       |  |
|                                     |   | 3              | 10685                    | 10450                  | 235              | 24                  | 1.2                       |                           |  |
| water control                       | 0   | 1              | 11125                    | 10894                  | 231              | 23                  | 0.0                       |                           |  |
|                                     |   | 2              | 11178                    | 10953                  | 225              | 23                  | 0.0                       | 0.0                       |  |
|                                     |   | 3              | 10523                    | 10294                  | 229              | 23                  | 0.0                       |                           |  |
| toxic standard<br>0.3 µg a.i./bee   | 0.015   | 1              | 10445                    | 10228                  | 217              | 22                  | 0.33                      |                           |  |
|                                     |   | 2              | 10512                    | 10281                  | 231              | 23                  | 0.35                      | 0.34                      |  |
|                                     |   | 3              | 10501                    | 10277                  | 224              | 22                  | 0.34                      |                           |  |
| toxic standard<br>0.15 µg a.i./bee  | 0.0075  | 1              | 10426                    | 10207                  | 219              | 22                  | 0.16                      |                           |  |
|                                     |   | 2              | 11187                    | 10957                  | 230              | 23                  | 0.17                      | 0.17                      |  |
|                                     |   | 3              | 10484                    | 10256                  | 228              | 23                  | 0.17                      |                           |  |
| toxic standard<br>0.075 µg a.i./bee | 0.0038  | 1              | 11149                    | 10920                  | 229              | 23                  | 0.09                      |                           |  |
|                                     |   | 2              | 11246                    | 11026                  | 220              | 22                  | 0.08                      | 0.09                      |  |
|                                     |   | 3              | 11264                    | 11037                  | 227              | 23                  | 0.09                      |                           |  |
| toxic standard<br>0.038 µg a.i./bee | 0.0019  | 1              | 11200                    | 10975                  | 225              | 23                  | 0.04                      |                           |  |
|                                     |   | 2              | 11227                    | 11003                  | 224              | 22                  | 0.04                      | 0.04                      |  |
|                                     |   | 3              | 11226                    | 10997                  | 229              | 23                  | 0.04                      |                           |  |

<sup>a</sup> weight of syringes at the start of the experiment, <sup>b</sup> after removing from the test cages; ingested solution as calculated average, <sup>d</sup> results are rounded results, calculated from the exact data

## **Annex: Test Item Approval**

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