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# Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology

## Report of the Validation Study for the Standardisation of a 10 day Feeding Test on Honey Bees

*The Joint Meeting is invited to agree to declassify the document by 10 July 2017.*

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**English - Or. English**

# REPORT OF THE VALIDATION STUDY FOR THE STANDARDISATION OF A 10 DAY FEEDING TEST ON HONEY BEES

This report was endorsed by the 29th Meeting of the WNT in April 2017.

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**PART 1**

# FINAL REPORT – SUMMARY OF THE RESULTS OF THE INTERNATIONAL RING TEST FOR THE STANDARDISATION OF A 10 DAY CHRONIC FEEDING TEST ON HONEY BEES (*APIS MELLIFERA* L.) IN THE LABORATORY

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## INTRODUCTION

1. Pollinators like honey bees (*Apis mellifera*) may be exposed to residues of plant protection products (PPP) for a prolonged period of time, *e.g.* via ingestion of contaminated food, stored and consumed by the bees in the hive or by foraging nectar and pollen of (seed) treated crops. To assess this potential risk a chronic toxicity test can be conducted in the laboratory. Here, young adult worker bees (1-4 days old) are continuously exposed to treated food over a period of 10 days.

2. According to EC Regulation 1107/2009, an active substance or PPP shall be approved only if it has no unacceptable acute or chronic effects on adult honey bees. Actual guidelines cover the acute effect of active substances or formulations on adult honey bees, but there is increasing need for further investigation of long term effects on honey bees after chronic oral exposure. Already available publications (Decourtye *et. al.* (2005), Suchail *et al.* (2001), CEB (2012)) describe methods that could possibly serve as testing procedure in an environmental risk assessment. However, none of these procedures/methods have been ring tested and validated yet.

3. In order to summarize practical experience and to harmonize the testing procedure, two independent ring tests were conducted in 2013 and 2014. In 2013, 6 German laboratories took part in this first ring test and the results were summarized in a separate report ("Summary of the Results of a Test Conducted by several German Laboratories for the Standardisation of a Test Design, November 15, 2013). Following this, an international OECD ring test group was founded in November 2013. During a first meeting on May 08, 2014, an international team of experts developed a protocol based on the experiences of the first ring test with modifications according to the experiences of the first ring test and based on the OECD TG 213. At this time some of the participating labs had already conducted these chronic toxicity tests for several years and collected valuable experiences with this test design.

4. In total 17 laboratories from 8 countries including two bee institutes, two industry laboratories and 13 contract labs participated in this working group in order to harmonize the current test procedures with the aim of the development of a Test Guideline for the evaluation of the chronic toxicity of PPP´s on adult honey bees in the lab.

5. This 10 day chronic feeding test should be used to cover a prolonged exposure phase and to determine endpoints like $LC_{50}$/$LDD_{50}$ (Lethal Concentration$_{50}$/Lethal Dietary Dose$_{50}$) and NOEC/NOEDD (No Observed Effect Concentration/No Observed Effect Dietary Dose), where possible.

6. The present data and experiences, generated and made by the ring test group allow setting of validity criteria for the controls as well as for the reference item treatment. Useful information on the climatic conditions, preparation of the honey bees and conduct of the test were obtained, enabling the set-up of a robust and standardized test guideline.

7. The chronic toxicity of the test item Perfekthion (a.i. dimethoate), known as the common toxic reference item for bee acute studies according to the OECD 213 guideline, was used as a reference item in this ring test. A number of 5 fixed concentrations of dimethoate (0.2, 0.4, 0.6, 0.8 and 1.0 mg a.i./kg feeding solution) were used in the ring test in order to determine $LC_{50}$/$LDD_{50}$ and NOEC/NOEDD values.

One stock solution was prepared and used for the whole test period and fresh feeding solutions were prepared at least every 4 days.

8. The stability of dimethoate in the feeding solutions, stored at 4 °C ± 2°C over a period of 10 days was assessed and confirmed, see chapter 8.6.

9. Two control groups were included in the test: one untreated control (50 % sugar solution) and a solvent control (50 % sugar solution containing 5 % acetone).

10. All bees were fed either with treated or untreated 50 % sugar solution (feeding solution).

11. Young adult honey bees (1 to 4 days old) were used in the test.

12. A validity criterion of ≤ 15 % mortality was set for both control groups taking into consideration the validity criterion mentioned in the EPPO 170 document as well as the OECD TG 213 with adoption to the prolonged test period.

13. In all participating laboratories the validity criterion was met for the untreated control group in all tests within the first run. However, for the solvent group (50 % sugar solution containing 5 % acetone) this validity criterion could not be met in some of the labs (mortality in these labs ranged from 16.7 % up to 90 %), indicating that the use of acetone as solvent at the tested concentration level is still questioned. For the reference item treatment group, mean $LC_{50}$ and $LDD_{50}$ values could be calculated, as well as the NOEC and NOEDD levels.

14. The tests were conducted under non-GLP conditions; however, many of the participating labs were GLP certified.

15. The results gained in these tests provided an indication of the suitability and reproducibility of the method which could serve as a basis for an official test guideline. However, in contracted studies which were conducted outside the ring test in the participating labs, problems were observed regarding the solubility especially of technical test item and the homogeneity of solutions prepared with formulated products.

## INFORMATION ON THE RING TEST GROUP

16. In total 17 laboratories from 8 countries participated in the ring test group:

| Laboratory | Responsible person | Address and contact details |
|---|---|---|
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Cells marked in gray indicating the leaders of the ring test group

| Laboratory | Responsible person | Address and contact details |
|---|---|---|
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## TIME SCHEDULE

| Activity | Scheduled date |
|---|---|
| Ring Test Meeting | May 08, 2014 |
| Experimental start | May 2014 |
| Experimental completion | July 2014 |
| Delivery of Report to OECD | December XX 2014 |

## MATERIALS AND METHODS

### Reference Item

| Reference Item | | | |
|---|---|---|---|
| Name / Code | Perfekthion / BAS 152 11 I | Batch number | FRE-000926 |
| Test item code | - | Appearance / colour | liquid / blue |
| Formulation type | EC | Intended usage | insecticide |
| Active ingredient | dimethoate | Content of a.i. nominal | 400 g/L |
| CAS number | 60-51-5 | Content of a.i. analysed | 400.9 g/L |
| Density analysed | 1.069 g/cm³ | Risk symbol(s) | Xn |
| Certificate of analysis | August 06, 2013 | Expiry date | June 17, 2015 |
| Stability in feeding solution | Stable for 10 days* | Storage conditions | ambient temperature (-10°C to +25°C) |

*according to analytical stability analysis by BASF SE and IBACON, 2014

17. In the control group the bees received an untreated 50 % (w/v) aqueous sugar solution *ad libitum.* An additional control group was fed *ad libitum* with 50 % (w/v) aqueous sugar solution containing 5 % acetone. The sugar solution was prepared with deionized water.

**Test Organism / Test System**

| | |
|---|---|
| Species/Variety | *Apis mellifera* L. (Hymenoptera, Apidae) |
| Life stage | Young adult worker bees (freshly hatched; 1 to 4 days old) |
| Source | Commercial supplier or own stock beehives. The source, including the name and address of the beekeeper, are documented in the ring test raw data. |
| Stock keeping | The hive(s) used for honeybee collection for this ring test were adequately fed, healthy and as far as possible disease-free and queen-right with known history and physiological status.<br>No chemical substances (such as antibiotics, anti-*Varroa* treatments, pesticides, *etc.*) were used in the hives for at least one month prior to this test.<br>Colony inspections and stock keeping of honeybees were recorded by the respective laboratories. |
| Collection of the bees | Honey bees were not older than 4 days at test start.<br>Brood combs from several colonies could be used to obtain the number of bees needed for the test. If so, it was suggested that the combs were combined during the hatching of the bees to ensure that the bees mixed naturally.<br>Two methods of collection of the bees were provided:<br><br><u>Method 1: Hatching in an incubator</u><br>Up to four days prior to test start, one or more brood comb(s) containing capped cells in which bees were visibly starting to emerge on the same day were taken out of a honeybee colony and transferred into a climatic chamber or incubator. Adult honey bees sitting on these combs were brushed off previously. The combs were kept under test conditions (33 ± 2 °C). In case the brood comb(s) did not contain enough honey and pollen as food for the hatched bees, an additional pollen and/or honey comb could have been placed beside the brood combs (This method was used by most of the labs).<br><br><u>Method 2: Hatching inside the hive</u><br>One or more brood combs with sealed brood were used in the test in which bees were visibly starting to emerge. The combs used should have also contained pollen and nectar as food for the freshly hatched bees. The combs were taken out of the hive and adult bees were swept out. Each comb was placed in an excluder box and was returned to the hive for one further day. The freshly hatched bees remained in the excluder box in the hive and were fed by nurse bees until they were collected for the test. On the following day (application day), the freshly emerged worker bees were taken out of the excluder box and transferred in the test units (cages) for the acclimatisation period. |
| Acclimatisation | Bees were acclimatized to test conditions for about one day. No additional feeding of pollen and water was supplied during acclimatization and test period.<br>Dead and moribund bees were rejected and replaced by healthy bees before test start. |

| | |
|---|---|
| | Care was taken by using suitable tools (*e.g.* forceps) to avoid damaging of the bees during the collection. |

**Test Design**

| | |
|---|---|
| Test design | Dose response test |
| Exposure time | 10 days |
| Number of treatment groups | 2 control groups (untreated and solvent control), 5 concentrations for the reference item group |
| Replicates per treatment group | 3 |
| Honey bees per treatment group | 30, divided in 3 replicates, each containing 10 test organisms Groups of 10 bees per cage were used, since this number allows a precise quantitative assessment of affected *vs.* non affected bees. |

**Test Cages**

| | |
|---|---|
| Test cages | The cages used were well-ventilated and made of material which was either easy to clean (reusable) or disposable.<br>Most of the labs used reusable test cages made of stainless steel.<br>But also test cages made of other materials (wood, cardboard, plastic) were used by participating laboratories. The size of the cages was appropriate to the number of bees *i.e.* providing adequate space.<br><br>Example for a steel cage:<br>The front side of the cage is equipped with a transparent pane to enable observation. The bottom of the cage consists of perforated steel, which guarantees sufficient air supply. The cages were lined with filter paper. |

**Test Conditions**

| | |
|---|---|
| Temperature | 33 ± 2°C |
| Relative humidity | 50 - 70 % |
| Exposure to light | Constant darkness except during the exchange of feeders and assessments |
| Ventilation | The test room/climatic chambers were ventilated. |
| Feeding | During acclimatisation: 50 % aqueous sugar solution *ad libitum.*<br>During test period: continuously *ad libitum* with treated or untreated feeding solutions |
| Specific documentation | Temperature and humidity were recorded continuously with appropriate, calibrated equipment.<br>(Short-term deviations (< 2 hours) from the recommended temperature and humidity ranges are unavoidable and should not affect the integrity or outcome of the study). |

**Feeding Solutions**

| | |
|---|---|
| Feeding solutions | The feeding solutions for the control and reference item treatments were prepared with 50 % (w/v) sugar solution. |
| Specific documentation | The bees were fed continuously *ad libitum* with a 50 % (w/v) sugar solution containing either:<br>• the reference item at 5 concentrations (reference item treatment group)<br>• pure 50 % (w/v) sugar solution (untreated control group),<br>• 5 % acetone as solvent (solvent control group).<br>The treated and untreated food was offered using plastic syringes or other suitable devices as feeders. Every day the feeder was replaced by a new one with fresh treated or untreated food. |

**Application**

***Preparation of the Control and Reference Item Feeding Solutions***

| | |
|---|---|
| Preparation of the control feeding solution | The feeding solution for the control group was prepared with 50 % (w/v) aqueous sugar solution.<br>For the additional solvent control the 50 % (w/v) aqueous sugar solution was mixed with 5 % acetone.<br>The control feeding solutions were prepared at least every 4 days and were stored in the refrigerator at 4 °C ± 4 °C. |
| Preparation of the reference item feeding solution(s) | A stock solution of the reference item was prepared once for the whole test period and stored in the refrigerator at 4 °C ± 4 °C for up to 10 days. Deionised water was used as the solvent.<br>The reference item feeding solutions were prepared out of the stock solution at least every 4 days and were stored in the refrigerator at 4°C ± 4°C.<br>[dimethoate is stable in sugar solution over a period of 10 days when stored in the refrigerator at 4 °C ± 4 °C; Internal BASF SE analytical report (March 25, 2014) and IBACON analytical report (79371136A, March 19, 2014)] |

***Application Details***

| | |
|---|---|
| Concentration levels | 0.2, 0.4, 0.6, 0.8 and 1.0 mg a.i. (dimethoate)/kg |
| Dose levels: | The exact dose per bee was calculated at the end of the test after determination of the actual food uptake. |
| Application mode | The treated and untreated feeding solutions were offered to the test organisms via feeders introduced into each test unit (*e.g.* plastic syringes, approx. 10 mL). If a syringe was used as a feeder, the tip of each syringe was removed so that the bees had access to the feeding solution. |
| Amount of feeding solution | The quantity of food offered to each cage should have exceeded the amount actually needed by the bees in order to guarantee an *ad libitum* feeding. The bees in one test unit shared the feeding solution and thus received similar doses (trophallaxis). |
| Application procedure | Every day (± 2 h in relation to the 1st application) the feeders containing the different control and reference item feeding solutions were replaced by fresh ones (one application interval). The treated and untreated feeding solutions were offered *ad libitum* to each cage in feeders. The feeders were weighed daily before introduction into the cages and after the feeding interval. |
| Dose verification | The amount of feeding solution(s) consumed was determined by weighing the feeders before and after feeding, using calibrated equipment. |

***Time Schedule***

| Time in relation to the first feeding | Action |
|---|---|

| | |
|---|---|
| Day - 4 to day -1 | Incubation of the brood combs |
| Day - 1 to day 0 | Collection of bees and acclimatisation |
| Day 0 = 1st Application | Preparation of stock solution(s), preparation of feeding solution(s), weighing of feeders; 1st application |
| Day 1 to day 9 (± 2 h in relation to 1st Application) | Assessment of mortality and behavioural abnormalities; preparation of feeding solutions (at least every four days), daily replacement of feeding solutions by fresh ones, determination of consumed feeding solutions by weighing the feeders. |
| Day 10 (± 2 h in relation to 1st Application) | Assessment of mortality and behavioural abnormalities; determination of consumed feeding solutions by weighing the feeders |

**Assessments**

***Mortality, Behavioural Abnormalities and Food Consumption***

Mortality

Mortality was assessed and recorded daily at about the same time of the day (every 24 h ± 2h), for a period of 10 days starting 24 ± 2 hours after start of the test period until test end.

Behavioural abnormalities

Behavioural abnormalities such as symptoms of poisoning or any abnormal behaviour in comparison to the control were recorded according to the following categories:

m = moribund (bees cannot walk and show only very feeble movements of legs and antennae, only weak response to stimulation; *e.g.* light or blowing; bees may recover but usually die),

a = affected (bees still upright and attempting to walk but showing signs of reduced coordination),

c = cramps (bees contracting abdomen or entire body),

ap = apathy (bees show only low or delayed reactions to stimulation *e.g.* light or blowing).

v = vomiting

Behavioural abnormalities were assessed and recorded daily at about the same time of the day (every 24 h ± 2h), for a period of 10 days and starting 24 ± 2 hours after start of the test period.

Food consumption

The food consumption per bee was calculated by the number of living bees at start of each feeding interval and the amount of food taken up until the following day.

Food consumption was assessed and recorded daily at about the same time of the day (every 24 h ± 2h) for a period of 10 days and, starting 24 ± 2 hours after start of the test period.

## DATA EVALUATION

### *Evaluation of Mortality and Behavioural Abnormalities*

18. Results obtained from the bees treated with the reference item or the solvent control was compared to those obtained from the control fed with pure sugar solution. The percentage of mortality was calculated for each treatment group from the number of dead individuals in correlation to the number of introduced test organisms.

The number of bees showing behavioural abnormalities were counted and given as absolute number/day in the results summary.

The data of the reference item and solvent control were not corrected for control mortality.

### *Evaluation of Food Consumption*

19. The weight of the8 feeders was recorded before and after feeding to the bees in order to calculate the exact food consumption per bee per day.

20. The difference of the weight before and after feeding to the bees represents the food consumed by the bees in one cage during one feeding interval (about 24 hours). This amount of food was divided by the number of living bees at the start of the corresponding feeding interval (*e.g.* day 4 – 5: consumed food at day 5 divided by the living bees at day 4).

21. The mean uptake of test item/bee is presented for each day as well as accumulated over the entire test period. The mean uptake of test item per bee per day over the whole testing period was calculated by summing up the daily uptake per bee and dividing by the relevant number of days.

### *Statistics*

22. Statistical calculations were made by using the statistical program ToxRat Professional 2.10.

23. Fisher's Exact Test (Bonferroni-Holms corrected, right-sided, $p \leq 0.05$) was used to evaluate whether there are significant differences between the mortality data of the untreated control and the test reference item treatment group for the determination of the NOEC and NOEDD based on mortality. Furthermore this test was used to compare the data on mortality between the untreated control and the solvent control.

24. $LDD_{50}$ (expressed in µg/bee/day) and $LC_{50}$ (expressed in mg/kg) values with 95 % confidence intervals of the reference item group were calculated by means of a probit analysis or by using the Weibull analysis. The data of Lab 12 could only be analysed by estimations using the binominal distribution.

Explanations:

25. $LC_{50}$ (median Lethal Concentration) is a statistically calculated concentration of a substance that can cause death in 50 % of the test organisms at the end of the test period. It is expressed in *e.g.* mg or µg active ingredient or formulated product per kg food.

26. $LDD_{50}$ (median Lethal Dietary Dose) is a statistically calculated dietary dose of a substance that can cause death in 50 % of the test organisms at the end of the test period. It is expressed in *e.g.* µg or ng active ingredient or formulated product per bee.

27. NOEC (No Observed Effect Concentration) the highest tested concentration with no statistically significant effect on the test organisms, when compared to the control. It is expressed in *e.g.* mg or µg active ingredient or formulated product per kg food.

28. NOEDD (No Observed Effect Dietary Dose) the highest tested dose per bee per day administered by chronic feeding exposure, with no statistically significant effect on the test organisms, when compared to the control. It is expressed in *e.g.* µg active ingredient or formulated product per bee per day.

## VALIDITY CRITERION

| | |
|---|---|
| Control mortality | The validity threshold for mean mortality in the control groups at the end of the test was set to ≤ 15 %.<br>This validity criterion was adopted from the validity criterion of the EPPO 170 guideline (≤ 15 %) and OECD TG 213 (≤ 10 %), by taking into consideration the prolonged test period of 10 days. |

## DEVIATIONS TO THE PROTOCOL

29. No major deviations were reported b the participating labs.

## RESULTS AND DISCUSSION

### Mortality Results of the Reference Item

30. TABLE 1 and TABLE 6 are presenting the cumulative mortality in the control group and the reference item group over the 10- day test period, diagrammed in FIGURE 1 and FIGURE 2.

**TABLE 1 Cumulative mortality [%] in the reference item treatment group during the 10 days test period**

| Treatment [mg a.i./kg] | Cumulative mortality [%] | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Lab 1** | **Lab 2** | **Lab 3** | **Lab 4** | **Lab 5** | **Lab 6** | **Lab 7** | **Lab 8** | **Lab 9** | **Lab 10** | **Lab 11** | **Lab 12** | **Lab 13** | **Lab 14** | **Lab 15** | **Lab 16** | **Lab 17** | **Mean** |
| **Untreated control*** | | | | | | | | | | | | | | | | | | |
| 0 | 6.7 | 0.0 | 6.7 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 0.0 | 6.7 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 0.0 | **2.0** |
| **Reference item: Perfekthion** | | | | | | | | | | | | | | | | | | |
| 0.2 | 3.3 | 6.7 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 0.0 | 10.0 | 26.7 | 0.0 | 36.7 | 10.0 | 6.7 | 6.7 | 0.0 | 3.3 | **6.9** |
| 0.4 | 0.0 | 13.3 | 16.7 | 20.0 | 60.0 | 80.0 | 50.0 | 26.7 | 80.0 | 70.0 | 26.7 | 100 | 10.0 | 36.7 | 13.3 | 0.0 | 30.0 | **37.3** |
| 0.6 | 40.0 | 40.0 | 73.3 | 56.7 | 93.3 | 100 | 100 | 33.3 | 100 | 73.3 | 100 | 100 | 20.0 | 96.7 | 26.7 | 16.7 | 100 | **68.8** |
| 0.8 | 100 | 100 | 100 | 66.7 | 100 | 100 | 90.6 | 93.3 | 100 | 100 | 100 | 100 | 73.3 | 100 | 76.7 | 33.3 | 100 | **90.2** |
| 1.0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 93.3 | 100 | 100 | 80.0 | 100 | **98.4** |

* untreated control group was fed with 50 % aqueous sucrose solution

31. Over all 17 laboratories a clear dose-response correlation could be observed in the reference item treatment. At the lowest concentration level of 0.2 mg a.i./kg the mortality ranged between 0 % and 36.7 % at day 10. In 15 out of 17 laboratories the mortality accounted to 100 % at the highest concentration level of 1.0 % after 10 days. The mean mortality levels over all labs were 6.9, 37.3, 68.8, 90.2 and 98.4 % following treatment with dimethoate concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 mg a.i./kg feeding solutions, respectively.

32. Due to the long test period of 10 days a timely dose response correlation can be observed at concentration levels causing more than 50 % mortality. As to a standardized test method, this observation justifies the testing of only one concentration of the reference item which results in a mortality of ≥ 50 % at the end of the test.

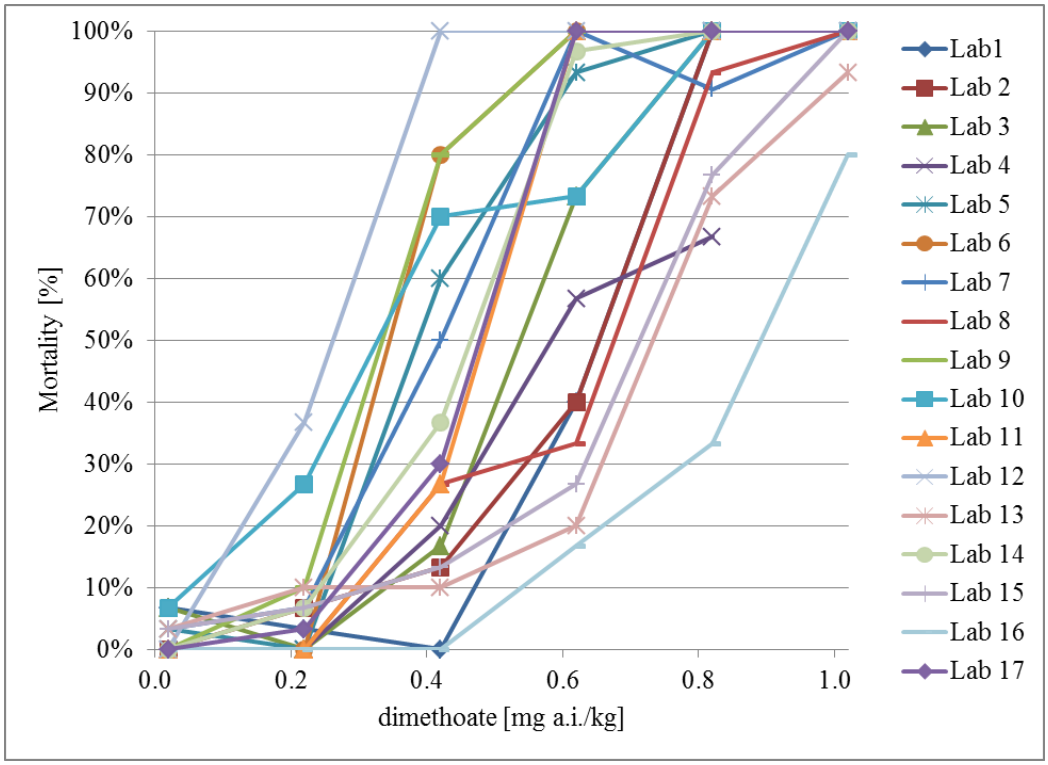


**FIGURE 1 Cumulative mortality in the reference item treatment group after 10 days**

**Mortality Results of the Control Groups**

33. The results of the cumulative mortality in both control groups (i.e. untreated and solvent control group) are presented in the following table:

**TABLE 2 Cumulative mortality [%] in the untreated and the solvent control group during the 10 days test period**

| Cumulative mortality [%] | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lab 1** | **Lab 2** | **Lab 3** | **Lab 4** | **Lab 5** | **Lab 6** | **Lab 7** | **Lab 8** | **Lab 9** | **Lab 10** | **Lab 11** | **Lab 12** | **Lab 13** | **Lab 14** | **Lab 15** | **Lab 16** | **Lab 17** | **Mean** | **Min** | **Max** |
| **Untreated control*** | | | | | | | | | | | | | | | | | | | |
| 6.7 | 0.0 | 6.7 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 0.0 | 6.7 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 0.0 | **2.0** | 0.0 | 6.7 |
| **Solvent control**** | | | | | | | | | | | | | | | | | | | |
| 3.3 | 6.7 | 3.3 | 6.7 | 20.0 | 6.7 | 90.0 | 3.3 | 6.7 | 16.7 | 3.3 | 80.0 | 0.0 | 16.7 | 33.3 | 0.0 | 23.3 | **18.8** | 0.0 | 90.0 |

* untreated control group was fed with 50 % aqueous sucrose solution

** solvent control group was fed with 50 % aqueous sucrose solution containing 5 % acetone

34. Two control groups were run concurrently in this ring test, one untreated 50 % w/v sugar control and one solvent control group with 50 % w/v sugar solution containing 5 % acetone.

35. For the untreated control, all labs met the internal validity criteria of ≤ 15 % within the first run. Mortality levels for the untreated control fed with pure 50 % w/v sugar solution ranged from 0.0 % to 6.7 %, resulting in a mean mortality level over all labs of 2.0 %. The validity criterion of ≤ 15 % was met by all labs and therefore all 17 tests were deemed valid.

36. Since many test items (mainly technical substances) are of low water solubility a suitable solvent should be available for this kind of test.

37. Therefore an additional solvent control group which was fed with 50 % w/v sugar solution containing 5 % acetone was included in the ring test. This solvent control group should have demonstrated that acetone is a suitable solvent also for chronic toxicity test and that a concentration of 5 % in the final feeding solution over a period of 10 days does not harm the bees.

38. However, the mortality in the solvent control group showed a great variability over the labs. The mortality levels ranged from 0.0 % to 90.0 %, resulting in a mean value over the labs of 18.8 %. In more than half of the tests (10 out of 17) the validity criterion of ≤ 15% was met also for the solvent control group. But in 7 out of 17 labs the mortality was beyond the accepted control mortality.

39. In order to find out more about the reasons of this unexpected high mortality in the solvent control group of some labs, some major parameters like e.g. bee race, acetone quality, age of bees, country of test conduct, were assessed. As a result of these investigations the following conclusions can be made:

- There is no indication for a bee race-related effect

- The acetone used had a high purity in all labs and was mostly of analytical quality
- No indication of a an effect related to the age of the tested bees
- No country specific reasons could be detected

40. In some labs a relation between the food uptake and the increased mortality was found. TABLE 6 shows a ranking of the laboratories based on the mean food uptake per bee per day over the test period. Four labs having the highest mean food uptake also showed an increased mortality beyond the validity criterion. Furthermore it was observed that in the respective labs the mortality did not continuously increase but started to rise mainly from day 6 onwards (see FIGURE 2). These two observations led to the assumption that there could be a certain threshold for the testing of acetone in some labs. This would mean that the bees are able to metabolize the acetone up to a specific level, but are affected or die as soon as this level has been exceeded.

41. To confirm this theory and furthermore to check for possible bee-age related effects, three additional tests were performed by IBACON and Eurofins:

42. Several concentrations of acetone in the feeding solutions were tested on young bees. The concentrations were equal or higher and the bees were of equal or lower age compared to the official ring test in order to have the worst case possible (see TABLE 3 to TABLE 5).

**TABLE 3 Mortality in solvent controls containing acetone at IBACON; age of bees at test start: 2 days**

| Treatment* | **Mortality [%]** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Test day | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | **0.0** |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | **0.0** |
| 7.5 % acetone | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 6.7 | **16.7** |
| 10 % acetone | 0.0 | 3.3 | 26.7 | 26.7 | 30.0 | 33.3 | 40.0 | 40.0 | 43.3 | **56.7** |

*bees were continuously fed either with pure 50% w/v sugar solution or with sugar solution containing the respective percentage of acetone; three replicates per treatment.

**TABLE 4 Mortality in solvent controls containing acetone at EAS; age of bees at test start: maximum 24 hours (no acclimatization)**

| Treatment* | **Mortality [%]** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Test day | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | **0.0** |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | **0.0** |
| 7 % acetone | 0.0 | 0.0 | 2.5 | 2.5 | - | 2.5 | 2.5 | 2.5 | 2.5 | **2.5** |
| 10 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | - | 0.0 | 5.0 | 10.0 | 15.0 | **20.0** |

*bees were continuously fed either with pure 50 % w/v sugar solution or with sugar solution containing the respective percentage of acetone; 4 replicates.
- no assessment was made on this day

**TABLE 5 Mortality in solvent controls containing acetone at EAS; age of bees at test start: 1-4 days**

| Treatment* | **Mortality [%]** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Test day | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | **0.0** |
| 5 % acetone | 0.0 | 0.0 | 2.5 | 2.5 | - | 2.5 | 2.5 | 2.5 | 7.5 | **7.5** |
| 7 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | - | 2.5 | 2.5 | 2.5 | 5.0 | **7.5** |
| 10 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | - | 5.0 | 10.0 | 15.0 | 22.5 | **35.0** |
| 12 % acetone | 5.0 | 27.5 | 37.5 | 37.5 | - | 57.5 | 72.5 | 75.0 | 87.5 | **87.5** |

*bees were continuously fed either with pure 50% w/v sugar solution or with sugar solution containing the respective percentage of acetone; 4 replicates per treatment.
- no assessment was made on this day

43. The bees for the additional studies presented in TABLE 4 and TABLE 5 were collected from the same combs, only at different times.

44. These experiments show a clear correlation between the concentration of acetone and mortality of the bees. Some experienced labs reported that they never observed mortalities above 15 % in the solvent control fed with 5 % acetone in several test starts beyond the actual ring test, whereas other labs observed increased mortality in the solvent control group of the ring test. Further investigations are needed to clarify the observed disparity.

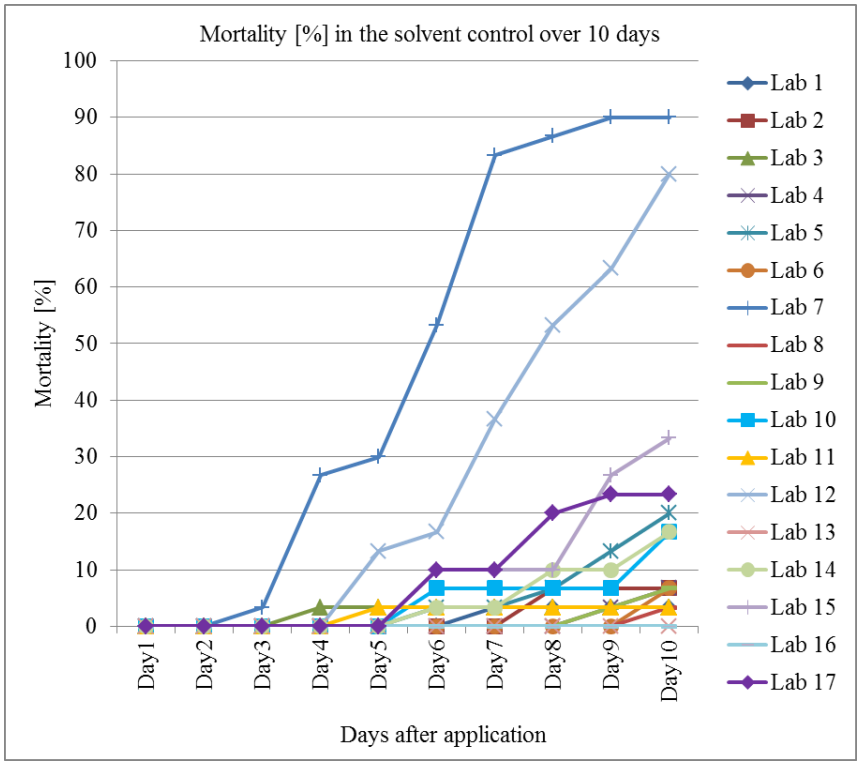


**FIGURE 2 Mortality [%] in the solvent control group over 10 days, treated with 50 % aqueous sucrose solution containing 5 % acetone**

**TABLE 6 Comparison of parameters differing between laboratories and possibly influencing mortality in the solvent control; the labs are ranked based on the food uptake [mg/bee/day] in the solvent control group**

| Lab 16 | Lab 1 | Lab 13 | Lab 17 | Lab 3 | Lab 15 | Lab 6 | Lab 11 | Lab 8 | Lab 4 | Lab 9 | Lab 10 | Lab 2 | Lab 12 | Lab 14 | Lab 5 | Lab 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean food uptake in the solvent control [mg/bee/day] | | | | | | | | | | | | | | | | |
| 19.7 | 32.7 | 33.4 | 34.0 | 34.3 | 34.4 | 36.5 | 39.5 | 39.6 | 40.7 | 42.2 | 42.5 | 44.4 | 50.0 | 50.9 | 58.0 | 58.0 |
| Mortality in % in the untreated control* | | | | | | | | | | | | | | | | |
| 0.0 | 6.7 | 3.3 | 0.0 | 6.7 | 3.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 |
| Mortality in % in the solvent control** | | | | | | | | | | | | | | | | |
| 0.0 | 3.3 | 0.0 | 23.3 | 3.3 | 33.3 | 6.7 | 3.3 | 3.3 | 6.7 | 6.7 | 16.7 | 6.7 | 80.0 | 16.7 | 20.0 | 90.0 |
| Purity of Acetone [%] | | | | | | | | | | | | | | | | |
| > 99.5 | 99.8 | 99.5 | ~100 | >99 | 99.8 | 99.9 | ~100 | >96 | > 95 | 99.8 | 99.9 | 99.7 | 99.5 | - | - | 99.8 |
| Bee race | | | | | | | | | | | | | | | | |
| ca | ca | ca | l | m | ca | b | b | l | l | ca | ca | ca | l | b | ca | ca |
| Age of bees at test start [d] | | | | | | | | | | | | | | | | |
| 1 - 4 | 2 | 1 - 4 | > 1 | 1 - 4 | 1 - 4 | 2 - 4 | 1 | 1 - 4 | 1 - 4 | 1 - 4 | 3 - 4 | 1 - 4 | 1 - 4 | 1 - 4 | 1 - 4 | 4 |
| Country of test conduct | | | | | | | | | | | | | | | | |
| D | D | D | I | E | D | F | GB | I | USA | NL | CH | D | USA | F | D | CH |

Columns marked in grey means that the validity criterion was not met
* untreated control group was fed with 50 % aqueous sucrose solution
** solvent control group was fed with 50 % aqueous sucrose solution containing 5 % acetone
- no information available
b = *Apis mellifera* buckfast
ca = *Apis mellifera* carnica
m = *Apis mellifera* mellifera
l = *Apis mellifera* ligustica

**Food consumption during the 10 days test period**

45. The results of the food consumption are presented in the following table:

TABLE 7 Mean consumption of feeding solution over the 10 days test period [mg/bee/day]

| Consumption of feeding solution [mg/bee/day] | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment** [mg a.i./kg] | **Lab 1** | **Lab 2** | **Lab 3** | **Lab 4** | **Lab 5** | **Lab 6** | **Lab 7** | **Lab 8** | **Lab 9** | **Lab 10** | **Lab 11** | **Lab 12** | **Lab 13** | **Lab 14** | **Lab 15** | **Lab 16** | **Lab 17** | **Mean** | **SD** |
| **Untreated control** | | | | | | | | | | | | | | | | | | | |
| 0 | 33.4 | 49.0 | 32.0 | 44.5 | 50.1 | 37.0 | 40.0 | 46.7 | 46.7 | 35.2 | 33.6 | 38.4 | 38.8 | 64.0 | 27.5 | 27.9 | 49.7 | **40.9** | 9.2 |
| **Solvent control**** | | | | | | | | | | | | | | | | | | | |
| 0 | 32.7 | 44.4 | 34.3 | 40.7 | 58.0 | 36.5 | 58.0 | 39.6 | 42.2 | 42.5 | 39.5 | 50.0 | 33.4 | 50.9 | 34.4 | 19.7 | 34.0 | **40.6** | 9.4 |
| **Reference item: Perfekthion** | | | | | | | | | | | | | | | | | | | |
| 0.2 | 33.0 | 40.5 | 28.8 | 35.7 | 42.2 | 33.4 | 36.1 | 39.7 | 42.0 | 36.0 | 26.7 | 39.0 | 31.2 | 46.7 | 29.1 | 29.0 | 34.6 | **35.5** | 5.4 |
| 0.4 | 27.0 | 30.2 | 28.5 | 29.3 | 38.9 | 33.2 | 32.1 | 36.7 | 43.5 | 36.9 | 22.6 | 36.5 | 27.0 | 38.9 | 23.5 | 26.4 | 29.6 | **31.8** | 5.8 |
| 0.6 | 23.8 | 29.3 | 27.8 | 28.0 | 51.2 | 41.2 | 33.4 | 32.6 | 50.2 | 28.6 | 27.6 | 43.3 | 26.6 | 41.7 | 20.5 | 20.6 | 38.0 | **33.2** | 9.2 |
| 0.8 | 32.4 | 38.8 | 37.5 | 31.5 | 48.1 | 32.1 | 35.4 | 37.9 | 40.9 | 30.7 | 25.4 | 24.8 | 30.1 | 35.3 | 28.1 | 19.4 | 55.5 | **34.3** | 8.4 |
| 1.0 | 23.7 | 30.9 | 34.0 | 35.7 | 41.2 | 32.8 | 41.3 | 40.3 | 50.9 | 31.5 | 24.7 | 36.5 | 29.4 | 35.9 | 26.2 | 18.7 | 49.7 | **34.3** | 8.5 |

* untreated control group was fed with 50 % aqueous sucrose solution
** solvent control group was fed with 50 % aqueous sucrose solution containing 5 % acetone

46. There was a distinct difference in food consumption of the bees among the laboratories, which ranged from 27.5 to 64.0 mg/bee/day for the untreated control group. The mean value over all 17 labs was 40.9 mg/bee ± 9.2. The same counts for the acetone control. Here, a mean value of 40.6 mg/bee ± 9.4 was found. The general comparable food uptake between the untreated and the solvent control group indicates that the solvent acetone has no anti-feeding or repellent effect.

47. The food consumption was slightly reduced in almost all labs and treatments during the first two test days (results not included in the Appendix).

48. There is no indication for a race-specific or country dependent relation of the food uptake.

49. A clear relationship could be demonstrated between the consumed test item dose and resulting mortality.

**TABLE 8 Accumulated mean uptake and related dose of reference item over 10 days [µg dimethoate/bee]**

| Treatment [mg a.i./kg] | Mean uptake of reference item [µg dimethoate/bee] | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Lab 1** | **Lab 2** | **Lab 3** | **Lab 4** | **Lab 5** | **Lab 6** | **Lab 7** | **Lab 8** | **Lab 9** | **Lab 10** | **Lab 11** | **Lab 12** | **Lab 13** | **Lab 14** | **Lab 15** | **Lab 16** | **Lab 17** | **Mean** | **SD** |
| Reference item: Perfekthion | | | | | | | | | | | | | | | | | | | |
| 0.2 | 0.007 | 0.008 | 0.006 | 0.007 | 0.008 | 0.007 | 0.007 | 0.008 | 0.008 | 0.007 | 0.005 | 0.008 | 0.006 | 0.009 | 0.006 | 0.006 | 0.007 | **0.007** | 0.001 |
| 0.4 | 0.011 | 0.012 | 0.011 | 0.012 | 0.016 | 0.013 | 0.012 | 0.015 | 0.017 | 0.015 | 0.009 | 0.015 | 0.011 | 0.016 | 0.009 | 0.011 | 0.012 | **0.013** | 0.002 |
| 0.6 | 0.014 | 0.018 | 0.017 | 0.017 | 0.031 | 0.025 | 0.020 | 0.020 | 0.030 | 0.017 | 0.017 | 0.026 | 0.016 | 0.025 | 0.014 | 0.012 | 0.023 | **0.020** | 0.005 |
| 0.8 | 0.026 | 0.031 | 0.030 | 0.025 | 0.035 | 0.026 | 0.028 | 0.030 | 0.037 | 0.025 | 0.020 | 0.026 | 0.024 | 0.028 | 0.023 | 0.016 | 0.044 | **0.028** | 0.006 |
| 1.0 | 0.023 | 0.031 | 0.043 | 0.036 | 0.041 | 0.033 | 0.039 | 0.038 | 0.051 | 0.031 | 0.025 | 0.033 | 0.029 | 0.036 | 0.026 | 0.019 | 0.037 | **0.034** | 0.008 |

## $LC_{50}$ and $LDD_{50}$

50. The results of the $LC_{50}$/10d, $LDD_{50}$/10d and NOEC/NOEDD values of dimethoate over 10 days are presented in the following table and figures:

**TABLE 9 $LC_{50}$, $LDD_{50}$ and NOEC/NOEDD values of dimethoate**

| **Lab 1** | **Lab 2** | **Lab 3** | **Lab 4** | **Lab 5** | **Lab 6** | **Lab 7** | **Lab 8** | **Lab 9** | **Lab 10** | **Lab 11** | **Lab 12** | **Lab 13** | **Lab 14** | **Lab 15** | **Lab 16** | **Lab 17** | **Mean** | **SD** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **$LC_{50}$** [mg a.i./kg] | | | | | | | | | | | | | | | | | | |
| 0.59 | 0.54 | 0.50 | 0.58 | 0.38 | 0.34 | 0.39 | 0.59 | 0.30 | 0.30 | 0.44 | 0.23 | 0.64 | 0.42 | 0.65 | 0.85 | 0.41 | **0.48** | 0.15 |
| Lower confidence limit | | | | | | | | | | | | | | | | | | |
| n.d. | n.d. | 0.46 | 0.51 | 0.34 | 0.22 | 0.34 | 0.27 | 0.26 | n.d. | 0.41 | 0.20 | n.d. | 0.04 | 0.07 | 0.79 | n.d. | **0.33** | 0.20 |
| Upper confidence limit | | | | | | | | | | | | | | | | | | |
| n.d. | n.d. | 0.55 | 0.64 | 0.42 | 0.38 | 0.43 | 0.75 | 0.34 | n.d. | 0.51 | 0.40 | n.d. | 0.46 | 0.87 | 0.91 | n.d. | **0.56** | 0.19 |
| **NOEC** [mg a.i./kg] | | | | | | | | | | | | | | | | | | |
| 0.40 | 0.40 | 0.40 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.60 | 0.20 | 0.40 | 0.60 | 0.20 | **0.28** | 0.15 |
| **$LDD_{50}$** [µg a.i./bee/day] | | | | | | | | | | | | | | | | | | |
| 0.015 | 0.019 | 0.014 | 0.018 | 0.016 | 0.011 | 0.011 | 0.02 | 0.013 | 0.011 | 0.01 | 0.009 | 0.019 | 0.017 | 0.017 | 0.017 | 0.013 | **0.015** | 0.003 |
| Lower confidence limit | | | | | | | | | | | | | | | | | | |
| n.d. | 0.017 | 0.013 | 0.016 | 0.014 | n.d. | 0.010 | 0.018 | 0.011 | 0.009 | 0.009 | 0.008 | 0.005 | 0.015 | 0.015 | 0.016 | 0.012 | **0.013** | 0.0036 |
| Upper confidence limit | | | | | | | | | | | | | | | | | | |
| n.d. | 0.021 | 0.016 | 0.020 | 0.018 | n.d. | 0.013 | 0.022 | 0.014 | 0.012 | 0.013 | 0.015 | 0.027 | 0.019 | 0.018 | 0.018 | 0.015 | **0.017** | 0.0039 |
| **NOEDD** [µg a.i./bee/day] | | | | | | | | | | | | | | | | | | |
| 0.011 | 0.012 | 0.011 | 0.007 | 0.008 | 0.007 | 0.007 | 0.008 | 0.008 | 0.007 | 0.005 | 0.008 | 0.016 | 0.009 | 0.009 | 0.012 | 0.007 | **0.009** | 0.0026 |

51. The $LC_{50}$ of dimethoate after 10 days was similar among the labs and ranged from 0.23 to 0.85 mg a.i./kg over the participating labs.

52. The resulting mean $LC_{50}$ value was 0.48 ± 0.15 mg a.i./kg. The mean $LDD_{50}$ over all laboratories was 0.015 µg a.i./bee ranging from 0.009 to 0.02 µg a.i./bee. Confidence limits could be calculated for most but not for all data sets.

53. NOEC and NOEDD values could be determined for all studies. Mean NOEC for dimethoate was 0.28 ± 0.15 mg a.i./kg and the mean NOEDD was 0.009 ± 0.0026 µg a.i./bee

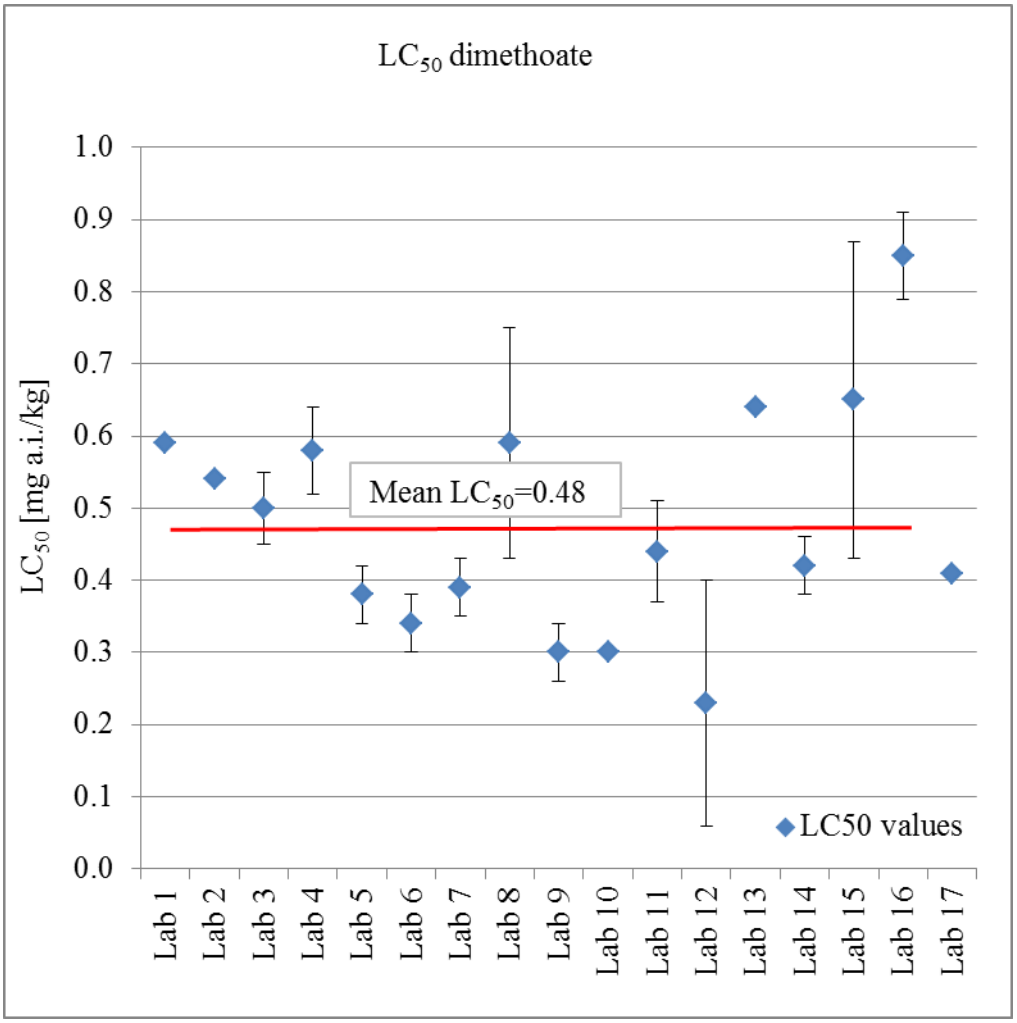


**FIGURE 3 $LC_{50}$ values of dimethoate after 10 days; mean value is visible in figure**

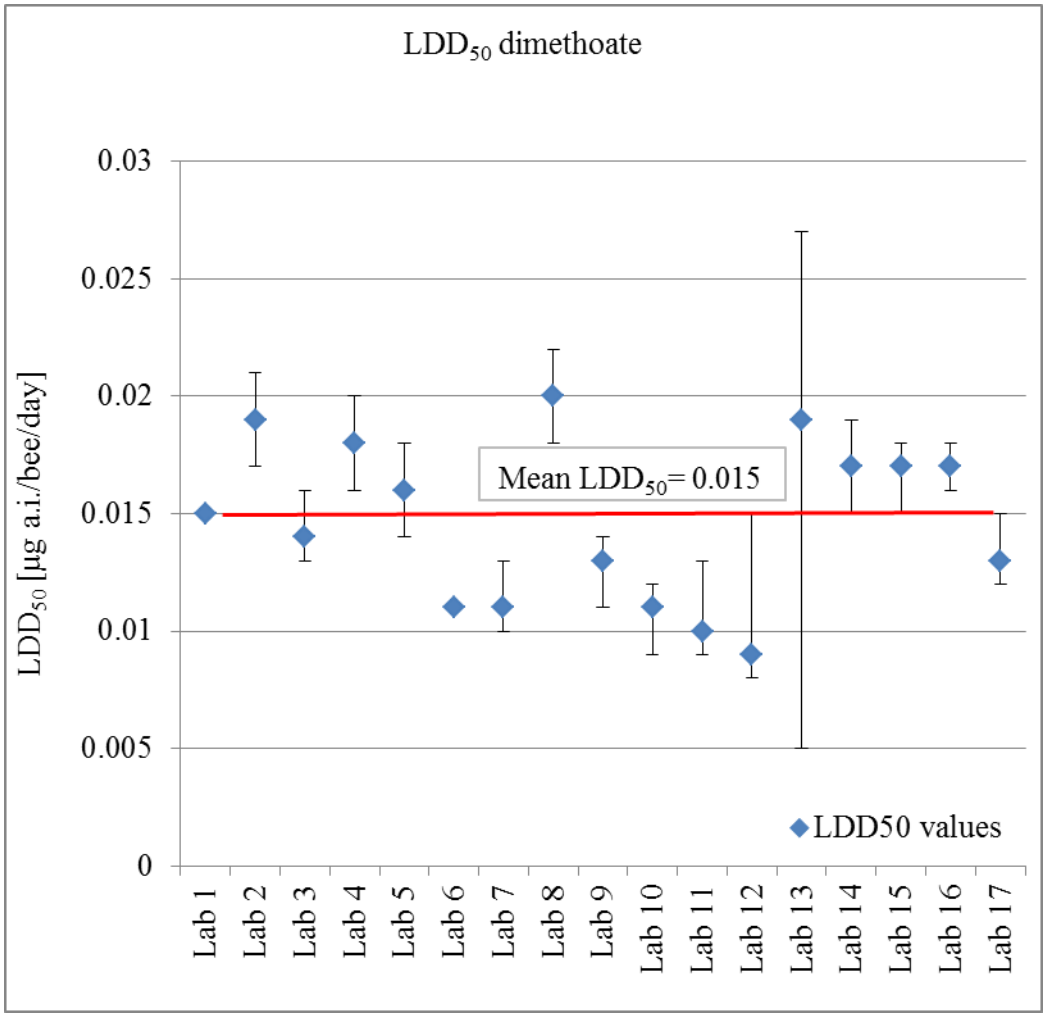


**FIGURE 4 $LDD_{50}$ values of dimethoate after 10 days; mean value is visible in figure**

**Behavioural Abnormalities**

54. Related to the effects caused by the dimethoate treatment, behavioural abnormalities occurred mainly at the higher concentration/dose levels. Most of these bees were categorised as affected, apathetic or moribund. These behavioural abnormalities were documented by 11 labs (Lab 1, 2, 3, 5, 6, 7, 10, 11, 12, 15 and 17). Results not included in the Appendix.

55. Hence, the chronic toxicity test can also be used to detect behavioural effects in a qualitative and quantitative manner.

**Determination of dimethoate in sugar solution**

56. In order to investigate the stability of dimethoate in sugar solution two independent chemical analyses were performed at IBACON GmbH and BASF SE.

57. The results of the chemical analysis and the stability of dimethoate are presented in the following:

**IBACON**

| | |
|---|---|
| Method: | The content of dimethoate (1.0 mg a.i./L) in a sucrose solution was determined after 0, 7 and 10 days storage at 4 °C ± 4 °C in the dark using HPLC with UV detection. |
| Results: | The dimethoate content was determined to be:<br>1.01 mg dimethoate/L after 0 days;<br>1.00 mg dimethoate/L after 7 days;<br>0.89 mg dimethoate/L after 10 days. |

**BASF SE**

| | |
|---|---|
| Method: | The content of dimethoate (1.0 mg a.i./L) in a sucrose solution was determined after 0, 7 and 10 days storage at 4 °C ± 4 °C in the dark using UHPLC-MS/MS. |
| Results: | The dimethoate content was determined to be:<br>1.08 mg dimethoate/L after 0 days;<br>1.09 mg dimethoate/L after 7 days;<br>1.10 mg dimethoate/L after 10 days. |

58. Therefore the feeding solution containing dimethoate at a concentration level of 1.0 mg a.i./L can be considered as stable if stored for 10 days in the refrigerator at 4 ° C ± 4 °C.

## ADDITIONAL INVESTIGATIONS

- The stability of dimethoate in the feeding solutions, stored at 4 °C ± 2°C over a period of 10 days was assessed and confirmed; see 7.6. Determination of dimethoate in sugar solution (2 studies conducted by Lab 1 IBACON GmbH and Lab 16 BASF SE)
- The size of the test cages has no influence on the outcome of the study. 3 studies (2 x Lab 5 BioChem agrar GmbH and 1 x Lab 7 Agroscope) were conducted with test cages which sizes deviated from the recommendations in the protocol
- The test material of the test cages (steel, plastic, cardboard) has no influence on the outcome of the study (based on the results of 2 studies conducted by Lab 7 Agroscope and Lab 17 CRA-Api)
- Sugar solution can be stored in the refrigerator for 4 days without any sign of bacterial or fungicidal contamination (one microbiological study, Lab 1 IBACON GmbH).
- Syringes are the most suitable feeders. (2 studies compared syringes with other items (e.g. Eppendorf cups); conducted in Lab 10 IES and Lab 7 Agroscope).
- Tests with solvent mediators (*e.g.* Tween20, Tween80) were done (> 3 studies, *e.g.* Lab 1 IBACON GmbH, *etc*.).
- Dimethylformamide (DMF) and Dimethylsulfoxid (DMSO) should not be used as solvent for chronic toxicity tests. One study conducted in Lab 9 Eurofins Mitox, showed that DMF causes high mortality (>20 %) even at low concentration levels (*e.g.* 0.04 % in the final feeding solution). Another study conducted in Lab 2 Eurofins showed that DMSO has an anti-feeding effect at the concentration level of 3 % in the final feeding solution
- Lower concentrated sugar solutions were tested (10, 15, 20 and 30 % w/v aqueous sucrose solution) to increase the solubility of test items with low water solubility. The results of these studies were heterogeneous and no conclusion could be made.

## CONCLUSIONS

59. In this ring test, 17 laboratories from 8 countries have conducted chronic toxicity tests on honey bees within a time frame of about 3 months in summer 2014. The labs followed a standardised protocol without main deviations. The aim of the ring test was, to show that under standardised conditions, this method can be used to gain reproducible and robust data on the toxicity of PPP`s in a chronic 10 day feeding test on honey bees.

60. A validity criterion of ≤ 15 % for the untreated control mortality was set and met by all laboratories. The observations of this ring test justify the testing of only one concentration of the reference item which results in a mortality of ≥ 50 % at the end of the test. Both validity criteria could be used in a standardized test guideline.

61. With the given test protocol and method, the aspired values like $LC_{50}$ and $LDD_{50}$, NOEC and NOEDD could be determined for all laboratories.

62. The results of these ring test showed, that the described test method is suitable to assess the chronic effects of plant protection products or other chemicals on honey bees in the laboratory. Two effective methods to obtain young adult worker honey bees with a known age and history were described, which are essentially needed to conduct a 10 day oral chronic feeding test. The test substance Perfekthion (a.i. dimethoate) is suitable to demonstrate the sensitivity of the test system in a chronic oral feeding test.

63. However, in contracted studies which were conducted outside the ring test in the participating labs, problems were observed regarding the solubility of technical test item and furthermore regarding the homogeneity during one feeding interval of solutions which prepared with formulated products. These problems led to technically limited highest concentrations.

64. The use of acetone as a solvent is still questioned as the results did not show a unique picture. Acetone can be used as a solvent as long as the above mentioned control mortality validity criterion is met. Further tests should be performed by the same laboratories in order to collect more data on possible effects of acetone on the honey bees e.g. a “dose response test” including lower concentrations of acetone during the next season (2015).

## REFERENCES

CEB (Commission des Essais Biologiques), 2012. Méthode d'évaluation des effets de toxicité aiguë et á court terme des préparations phytopharmaceutiques sur l'abeille domestique (Apis mellifera L.). AFPP method n°230.

Decourtye, A. et al., 2005: Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honey bee Apis mellifera. Archives of Environmental Contamination and Toxicology, 48, 242-250.

Niederdrenk, S., Oppinger, M., Kusebauch, B., BASF SE internal Report, 2014: Determination of Dimethoate in 50 % Sucrose Solution

OECD 213, 1998 Guideline for the testing of chemicals; Honey bees; acute oral toxicity test.

Schmitzer, S, Fieseler, A., IBACON internal Report, 2014: Determination of Dimethoate in 50 % Sucrose Solution

Schneider-Orelli, O. (1947): Entomologisches Praktikum. H.R. Sauerländer & Co., Aarau, 2. Auflage.

Suchail, S., Guez, D. and Belzunces, LP., (2001): Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in Apis mellifera. Environmental Toxicology and Chemistry, 20, 2482-2486.

Report – Summary of the Results of a Test Conducted by several German Laboratories for the Standardisation of a Test Design (2013); Assessment of Chronic Effects to the Honeybee, *Apis mellifera* L., in a 10 Day Continuous Laboratory Feeding Test; compiled by Annette Kling, Stephan Schmitzer, Markus Barth, Dorothee Lüken, Nicole Hanewald, David Gladbach on behalf of the bee expert group „AG Bienenschutz", Germany

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# APPENDIX

**TABLE 10 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 1: IBACON GmbH**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality mean | day 1 behav. abnorm. mean | day 2 mortality mean | day 2 behav. abnorm. mean | day 3 mortality mean | day 3 behav. abnorm. mean | day 4 mortality mean | day 4 behav. abnorm. mean | day 5 mortality mean | day 5 behav. abnorm. mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | % | % | % | % | % | % | % | % | % | % |
| dimethoate | 1.0 | 0.023 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 33.3 | 0.0 |
| | 0.8 | 0.026 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 6.7 | 0.0 | 13.3 | 0.0 |
| | 0.6 | 0.014 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |
| | 0.4 | 0.011 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.2 | 0.007 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality mean | day 6 behav. abnorm. mean | day 7 mortality mean | day 7 behav. abnorm. mean | day 8 mortality mean | day 8 behav. abnorm. mean | day 9 mortality mean | day 9 behav. abnorm. mean | day 10 mortality mean | day 10 behav. abnorm. mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | % | % | % | % | % | % | % | % | % | % |
| dimethoate | 1.0 | 0.023 | 70.0 | 10.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.026 | 30.0 | 0.0 | 40.0 | 23.3 | 76.7 | 10.0 | 93.3 | 6.7 | 100.0 | 0.0 |
| | 0.6 | 0.014 | 6.7 | 0.0 | 16.7 | 3.3 | 23.3 | 6.7 | 33.3 | 13.3 | 40.0 | 16.7 |
| | 0.4 | 0.011 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10.0 | 0.0 | 13.3 | 0.0 | 13.3 |
| | 0.2 | 0.007 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |
| water control | 0.0 | - | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities

**TABLE 11 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 2: Eurofins, Germany**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.031 | 0.0 | 0.0 | 0.0 | 23.3 | 16.7 | 16.7 | 46.7 | 10.0 | 56.7 | 16.7 |
| | 0.8 | 0.031 | 0.0 | 0.0 | 0.0 | 13.3 | 16.7 | 6.7 | 53.3 | 10.0 | 56.7 | 23.3 |
| | 0.6 | 0.018 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |
| | 0.4 | 0.012 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |
| | 0.2 | 0.0081 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 6.7 | 0.0 | 6.7 | 3.3 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.031 | 73.3 | 13.3 | 93.3 | 6.7 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.031 | 63.3 | 26.7 | 83.3 | 16.7 | 90.0 | 3.3 | 90.0 | 10.0 | 100.0 | 0.0 |
| | 0.6 | 0.018 | 6.7 | 0.0 | 6.7 | 20.0 | 20.0 | 3.3 | 23.3 | 16.7 | 40.0 | 16.7 |
| | 0.4 | 0.012 | 6.7 | 0.0 | 10.0 | 13.3 | 10.0 | 3.3 | 10.0 | 13.3 | 13.3 | 20.0 |
| | 0.2 | 0.0081 | 6.7 | 3.3 | 6.7 | 13.3 | 6.7 | 0.0 | 6.7 | 3.3 | 6.7 | 3.3 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 12 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 3: Eurofins, Spain**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.042 | 0.0 | 0.0 | 0.0 | 0.0 | 10.0 | 23.3 | 20.0 | 20.0 | 53.3 | 46.7 |
| | 0.8 | 0.030 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 20.0 | 30.0 | 70.0 |
| | 0.6 | 0.017 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 96.7 |
| | 0.4 | 0.011 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.2 | 0.0058 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.042 | 80.0 | 20.0 | 93.3 | 3.3 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.030 | 36.7 | 20.0 | 53.3 | 16.7 | 70.0 | 30.0 | 96.7 | 3.3 | 100.0 | 0.0 |
| | 0.6 | 0.017 | 3.3 | 10.0 | 16.7 | 6.7 | 26.7 | 53.3 | 43.3 | 56.7 | 73.3 | 13.3 |
| | 0.4 | 0.011 | 0.0 | 3.3 | 6.7 | 0.0 | 6.7 | 0.0 | 10.0 | 60.0 | 16.7 | 16.7 |
| | 0.2 | 0.0058 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 0.0 |
| water control | 0.0 | - | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 |
| solvent control | 0.0 | - | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 13 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 4: Eurofins, USA**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.036 | 0.0 | 0.0 | 0.0 | 0.0 | 23.3 | 0.0 | 50.0 | 0.0 | 60.0 | 0.0 |
| | 0.8 | 0.025 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 23.3 | 0.0 | 40.0 | 0.0 |
| | 0.6 | 0.017 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 13.3 | 0.0 | 20.0 | 0.0 |
| | 0.4 | 0.012 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 0.0 |
| | 0.2 | 0.0071 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.036 | 70.0 | 0.0 | 86.7 | 0.0 | 90.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.025 | 43.3 | 0.0 | 50.0 | 0.0 | 56.7 | 0.0 | 66.7 | 0.0 | 66.7 | 0.0 |
| | 0.6 | 0.017 | 23.3 | 0.0 | 33.3 | 0.0 | 36.7 | 0.0 | 43.3 | 0.0 | 56.7 | 0.0 |
| | 0.4 | 0.012 | 10.0 | 0.0 | 13.3 | 0.0 | 13.3 | 0.0 | 20.0 | 0.0 | 20.0 | 0.0 |
| | 0.2 | 0.0071 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 6.7 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 14 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 5: BioChem Agrar, Germany**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.041 | 0.0 | 0.0 | 0.0 | 0.0 | 30.0 | 0.0 | 90.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.035 | 0.0 | 0.0 | 0.0 | 0.0 | 23.3 | 0.0 | 56.7 | 0.0 | 76.7 | 3.3 |
| | 0.6 | 0.031 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 16.7 | 0.0 | 30.0 | 10.0 |
| | 0.4 | 0.016 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 |
| | 0.2 | 0.0084 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.041 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.035 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.6 | 0.031 | 66.7 | 0.0 | 86.7 | 0.0 | 90.0 | 0.0 | 90.0 | 0.0 | 93.3 | 0.0 |
| | 0.4 | 0.016 | 16.7 | 0.0 | 26.7 | 0.0 | 36.7 | 0.0 | 53.3 | 0.0 | 60.0 | 0.0 |
| | 0.2 | 0.0084 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 0.0 | 13.3 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 |
| solvent control | 0.0 | - | 3.3 | 0.0 | 3.3 | 0.0 | 6.7 | 0.0 | 13.3 | 0.0 | 20.0 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 15 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 6: ACTA, France**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.033 | 0.0 | 0.0 | 6.7 | 0.0 | 16.7 | 0.0 | 46.7 | 20.0 | 96.7 | 3.3 |
| | 0.8 | 0.026 | 3.3 | 0.0 | 3.3 | 0.0 | 13.3 | 0.0 | 43.3 | 3.3 | 93.3 | 6.7 |
| | 0.6 | 0.025 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 10.0 | 10.0 | 26.7 | 16.7 |
| | 0.4 | 0.013 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10.0 | 0.0 |
| | 0.2 | 0.0067 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.033 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.026 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.6 | 0.025 | 76.7 | 10.0 | 90.0 | 10.0 | 96.7 | 0.0 | 96.7 | 3.3 | 100.0 | 0.0 |
| | 0.4 | 0.013 | 23.3 | 13.3 | 43.3 | 10.0 | 63.3 | 13.3 | 73.3 | 3.3 | 80.0 | 3.3 |
| | 0.2 | 0.0067 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 16 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 7: Agroscope, Switzerland**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.039 | 0.0 | 0.0 | 3.3 | 6.7 | 16.7 | 53.3 | 40.0 | 13.3 | 66.7 | 30.0 |
| | 0.8 | 0.028 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.1 | 6.3 | 43.8 | 12.5 | 34.4 |
| | 0.6 | 0.020 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 13.8 | 51.7 |
| | 0.4 | 0.0116 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 10.0 | 0.0 |
| | 0.2 | 0.0072 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 |
| water control[rep] | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control[rep] | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 26.7 | 0.0 | 30.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.039 | 80.0 | 0.0 | 83.3 | 6.7 | 93.3 | 6.7 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.028 | 31.3 | 37.5 | 46.9 | 40.6 | 81.3 | 18.8 | 96.9 | 3.1 | 100.0 | 0.0 |
| | 0.6 | 0.020 | 20.7 | 58.6 | 31.0 | 58.6 | 41.4 | 55.2 | 65.5 | 37.9 | 100.0 | 3.4 |
| | 0.4 | 0.0116 | 10.0 | 0.0 | 40.0 | 10.0 | 40.0 | 13.3 | 46.7 | 23.3 | 50.0 | 13.3 |
| | 0.2 | 0.0072 | 6.7 | 13.3 | 6.7 | 10.0 | 6.7 | 3.3 | 6.7 | 3.3 | 6.7 | 0.0 |
| water control | 0.0 | - | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |
| solvent control | 0.0 | - | 3.3 | 0.0 | 13.3 | 13.3 | 33.3 | 0.0 | 66.7 | 3.3 | 66.7 | 0.0 |
| water control[rep] | 0.0 | - | 0.0 | 0.0 | 6.7 | 0.0 | 10.0 | 0.0 | 13.3 | 0.0 | 13.3 | 0.0 |
| solvent control[rep] | 0.0 | - | 53.3 | 0.0 | 83.3 | 0.0 | 86.7 | 0.0 | 90.0 | 0.0 | 90.0 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or control s
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application
[rep] test with solvent control was repeated

**TABLE 17 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 8: BIOTECNOLOGIE BT, Italy**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.038 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 0.0 | 16.7 | 0.0 | 53.3 | 0.0 |
| | 0.8 | 0.030 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.6 | 0.020 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.4 | 0.015 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.2 | 0.008 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.038 | 70.0 | 0.0 | 86.7 | 0.0 | 93.3 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.030 | 20.0 | 0.0 | 56.7 | 0.0 | 73.3 | 0.0 | 93.3 | 0.0 | 93.3 | 0.0 |
| | 0.6 | 0.020 | 3.3 | 0.0 | 13.3 | 0.0 | 30.0 | 0.0 | 33.3 | 0.0 | 33.3 | 0.0 |
| | 0.4 | 0.015 | 0.0 | 0.0 | 3.3 | 0.0 | 23.3 | 0.0 | 23.3 | 0.0 | 26.7 | 0.0 |
| | 0.2 | 0.008 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 18 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 9: MITOX, the Netherlands**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.051 | 0.0 | 0.0 | 0.0 | 3.3 | 23.3 | 3.3 | 96.7 | 6.7 | 100.0 | 6.7 |
| | 0.8 | 0.037 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 33.3 | 20.0 | 96.7 | 20.0 |
| | 0.6 | 0.030 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 13.3 | 0.0 | 30.0 | 10.0 |
| | 0.4 | 0.0174 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 |
| | 0.2 | 0.0084 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.0509 | 100.0 | 6.7 | 100.0 | 6.7 | 100.0 | 6.7 | 100.0 | 6.7 | 100.0 | 6.7 |
| | 0.8 | 0.0370 | 100.0 | 20.0 | 100.0 | 20.0 | 100.0 | 20.0 | 100.0 | 20.0 | 100.0 | 20.0 |
| | 0.6 | 0.0301 | 63.3 | 13.3 | 90.0 | 16.7 | 100.0 | 16.7 | 100.0 | 16.7 | 100.0 | 16.7 |
| | 0.4 | 0.0174 | 6.7 | 6.7 | 13.3 | 6.7 | 33.3 | 13.3 | 63.3 | 20.0 | 80.0 | 20.0 |
| | 0.2 | 0.0084 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 6.7 | 0.0 | 10.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 6.7 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 19 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 10: IES, Switzerland**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.031 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 26.7 | 30.0 | 3.3 | 66.7 | 33.3 |
| | 0.8 | 0.025 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20.0 | 80.0 |
| | 0.6 | 0.017 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 33.3 | 66.7 |
| | 0.4 | 0.015 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 93.3 |
| | 0.2 | 0.0072 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 96.7 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 6.7 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.031 | 90.0 | 10.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.025 | 46.7 | 46.7 | 80.0 | 20.0 | 90.0 | 10.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.6 | 0.017 | 43.3 | 46.7 | 46.7 | 53.3 | 63.3 | 36.7 | 66.7 | 0.0 | 73.3 | 0.0 |
| | 0.4 | 0.015 | 50.0 | 50.0 | 50.0 | 50.0 | 53.3 | 46.7 | 60.0 | 0.0 | 70.0 | 10.0 |
| | 0.2 | 0.0072 | 3.3 | 0.0 | 6.7 | 0.0 | 10.0 | 0.0 | 10.0 | 0.0 | 26.7 | 0.0 |
| water control | 0.0 | - | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 |
| solvent control | 0.0 | - | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 16.7 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 20 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 11: FERA, UK**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.025 | 0.0 | 0.0 | 0.0 | 0.0 | 16.7 | 3.3 | 66.7 | 20.0 | 100.0 | 0.0 |
| | 0.8 | 0.020 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 20.0 | 10.0 | 50.0 | 16.7 |
| | 0.6 | 0.017 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10.0 | 0.0 |
| | 0.4 | 0.009 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.2 | 0.005 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.0247 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.0203 | 93.3 | 3.3 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.6 | 0.0166 | 16.7 | 23.3 | 63.3 | 16.7 | 86.7 | 10.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.4 | 0.0090 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 6.7 | 13.3 | 3.3 | 26.7 | 3.3 |
| | 0.2 | 0.0053 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 21 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 12: Wildlife International, USA**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.033 | 0.0 | 0.0 | 0.0 | 0.0 | 53.3 | 0.0 | 83.3 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.026 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 43.3 | 3.3 | 90.0 | 0.0 |
| | 0.6 | 0.026 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 33.3 | 0.0 | 70.0 | 0.0 |
| | 0.4 | 0.0146 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 0.0 |
| | 0.2 | 0.0078 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 13.3 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.033 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.026 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.6 | 0.026 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.4 | 0.015 | 23.3 | 0.0 | 53.3 | 0.0 | 80.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.2 | 0.0078 | 0.0 | 0.0 | 6.7 | 0.0 | 30.0 | 3.3 | 33.3 | 0.0 | 36.7 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 16.7 | 0.0 | 36.7 | 0.0 | 53.3 | 0.0 | 63.3 | 3.3 | 80.0 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or control
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 22 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 13: BAYER, Germany**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.029 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 0.0 |
| | 0.8 | 0.024 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 10.0 | 0.0 |
| | 0.6 | 0.016 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.4 | 0.0108 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 |
| | 0.2 | 0.0062 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 10.0 | 0.0 | 10.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.029 | 13.3 | 0.0 | 23.3 | 0.0 | 63.3 | 0.0 | 80.0 | 0.0 | 93.3 | 0.0 |
| | 0.8 | 0.024 | 16.7 | 0.0 | 40.0 | 0.0 | 56.7 | 0.0 | 73.3 | 0.0 | 73.3 | 0.0 |
| | 0.6 | 0.016 | 3.3 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 20.0 | 0.0 |
| | 0.4 | 0.011 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 10.0 | 0.0 | 10.0 | 0.0 |
| | 0.2 | 0.0062 | 10.0 | 0.0 | 10.0 | 0.0 | 10.0 | 0.0 | 10.0 | 0.0 | 10.0 | 0.0 |
| water control | 0.0 | - | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 23 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 14: Testapi, France**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.036 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.1 | 0.0 | 21.9 | 0.0 |
| | 0.8 | 0.028 | 0.0 | 0.0 | 0.0 | 0.0 | 12.9 | 0.0 | 19.4 | 0.0 | 38.7 | 0.0 |
| | 0.6 | 0.025 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 |
| | 0.4 | 0.0156 | 0.0 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 |
| | 0.2 | 0.0093 | 0.0 | 0.0 | 10.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.0359 | 59.4 | 0.0 | 84.4 | 0.0 | 90.6 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.0282 | 48.4 | 0.0 | 77.4 | 0.0 | 93.5 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.6 | 0.0250 | 10.0 | 0.0 | 23.3 | 0.0 | 46.7 | 0.0 | 90.0 | 0.0 | 96.7 | 0.0 |
| | 0.4 | 0.0156 | 10.0 | 0.0 | 13.3 | 0.0 | 23.3 | 0.0 | 26.7 | 0.0 | 36.7 | 0.0 |
| | 0.2 | 0.0093 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 3.3 | 0.0 | 3.3 | 0.0 | 10.0 | 0.0 | 10.0 | 0.0 | 16.7 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 24 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 15: LAVES, Germany**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.026 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.8 | 0.023 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 0.0 | 16.7 | 0.0 |
| | 0.6 | 0.014 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 |
| | 0.4 | 0.0094 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 |
| | 0.2 | 0.0058 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.0262 | 16.7 | 0.0 | 36.7 | 0.0 | 76.7 | 13.3 | 93.3 | 6.7 | 100.0 | 0.0 |
| | 0.8 | 0.0225 | 23.3 | 0.0 | 33.3 | 0.0 | 40.0 | 6.7 | 70.0 | 3.3 | 76.7 | 0.0 |
| | 0.6 | 0.0140 | 13.3 | 0.0 | 16.7 | 0.0 | 23.3 | 3.3 | 26.7 | 0.0 | 26.7 | 0.0 |
| | 0.4 | 0.0094 | 6.7 | 3.3 | 6.7 | 0.0 | 6.7 | 0.0 | 10.0 | 0.0 | 13.3 | 0.0 |
| | 0.2 | 0.0058 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 |
| solvent control | 0.0 | - | 10.0 | 0.0 | 10.0 | 0.0 | 10.0 | 0.0 | 26.7 | 0.0 | 33.3 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 25 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 16: BASF SE, Germany**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality mean | day 1 behav. abnorm. mean | day 2 mortality mean | day 2 behav. abnorm. mean | day 3 mortality mean | day 3 behav. abnorm. mean | day 4 mortality mean | day 4 behav. abnorm. mean | day 5 mortality mean | day 5 behav. abnorm. mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | % | % | % | % | % | % | % | % | % | % |
| dimethoate | 1.0 | 0.019 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.8 | 0.016 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.6 | 0.012 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.4 | 0.011 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.2 | 0.006 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality mean | day 6 behav. abnorm. mean | day 7 mortality mean | day 7 behav. abnorm. mean | day 8 mortality mean | day 8 behav. abnorm. mean | day 9 mortality mean | day 9 behav. abnorm. mean | day 10 mortality mean | day 10 behav. abnorm. mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | % | % | % | % | % | % | % | % | % | % |
| dimethoate | 1.0 | 0.019 | 10.0 | 0.0 | 20.0 | 0.0 | 46.7 | 0.0 | 70.0 | 0.0 | 80.0 | 0.0 |
| | 0.8 | 0.016 | 0.0 | 0.0 | 6.7 | 0.0 | 16.7 | 0.0 | 30.0 | 0.0 | 33.3 | 0.0 |
| | 0.6 | 0.012 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 16.7 | 0.0 |
| | 0.4 | 0.011 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.2 | 0.0058 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 26 Cumulative mortality in the control and the test item treatment groups during the 10 days test period;**
**Lab 17: CRA API, Italy**

| treatment group | concentration | mean dose per/bee/day* | day 1 mortality | day 1 behav. abnorm. | day 2 mortality | day 2 behav. abnorm. | day 3 mortality | day 3 behav. abnorm. | day 4 mortality | day 4 behav. abnorm. | day 5 mortality | day 5 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.037 | 0.0 | 0.0 | 10.0 | 16.7 | 66.7 | 26.7 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.044 | 0.0 | 3.3 | 3.3 | 0.0 | 6.7 | 0.0 | 53.3 | 36.7 | 96.7 | 0.0 |
| | 0.6 | 0.023 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 10.0 | 16.7 |
| | 0.4 | 0.012 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.2 | 0.007 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 6.7 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| treatment group | concentration | mean dose per/bee/day* | day 6 mortality | day 6 behav. abnorm. | day 7 mortality | day 7 behav. abnorm. | day 8 mortality | day 8 behav. abnorm. | day 9 mortality | day 9 behav. abnorm. | day 10 mortality | day 10 behav. abnorm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [mg a.i./kg] | [µg a.i./bee] | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % | mean % |
| dimethoate | 1.0 | 0.037 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.8 | 0.044 | 66.7 | 3.3 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| | 0.6 | 0.023 | 13.3 | 10.0 | 20.0 | 6.7 | 73.3 | 6.7 | 90.0 | 6.7 | 100.0 | 0.0 |
| | 0.4 | 0.012 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 13.3 | 3.3 | 30.0 | 13.3 |
| | 0.2 | 0.007 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 0.0 | 3.3 | 6.7 | 3.3 | 0.0 |
| water control | 0.0 | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| solvent control | 0.0 | - | 10.0 | 36.7 | 10.0 | 0.0 | 20.0 | 16.7 | 23.3 | 3.3 | 23.3 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or controls
behav. abnorm. = behavioural abnormalities
* at test end, 10 days following application

**TABLE 27 Accumulated Food Consumption in the untreated control group during the 10 days test period;**

**Accumulated Food Consumption in the Untreated Control Group**

| | day 0-1 | day 1-2 | day 2-3 | day 3-4 | day 4-5 | day 5-6 | day 6-7 | day 7-8 | day 8-9 | day 9-10 | Mean | Country | *Apis mellifera* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lab 1 | 22.5 | 27.3 | 31.1 | 29.4 | 34.9 | 41.3 | 36.6 | 39.6 | 37.7 | 33.4 | **33.4** | **D** | *carnica* |
| Lab 2 | 19.6 | 42.9 | 48.9 | 48.4 | 46.2 | 62.0 | 53.9 | 57.4 | 53.5 | 57.2 | **49.0** | **D** | *carnica* |
| Lab 3 | 36.9 | 26.4 | 19.6 | 29.2 | 31.8 | 30.3 | 38.0 | 34.0 | 33.8 | 40.3 | **32.0** | **E** | *mellifea* |
| Lab 4 | 39.8 | 46.9 | 47.2 | 55.8 | 38.4 | 42.4 | 50.8 | 45.5 | 41.3 | 36.8 | **44.5** | **USA** | *ligustica* |
| Lab 5 | 27.8 | 38.7 | 44.5 | 47.9 | 44.8 | 51.2 | 58.7 | 70.6 | 53.8 | 62.8 | **50.1** | **D** | *carnica* |
| Lab 6 | 26.3 | 40.6 | 26.6 | 37.1 | 38.5 | 41.8 | 37.1 | 35.4 | 43.0 | 43.5 | **37.0** | **F** | *buckfast* |
| Lab 7 | 23.3 | 32.7 | 43.4 | 33.6 | 34.6 | 46.7 | 48.9 | 40.7 | 46.3 | 50.1 | **40.0** | **CH** | |
| Lab 8 | 34.6 | 40.6 | 33.0 | 30.8 | 41.9 | 37.9 | 52.1 | 63.0 | 69.9 | 62.9 | **46.7** | **I** | *ligustica* |
| Lab 9 | 45.4 | 51.7 | 52.9 | 56.2 | 47.9 | 44.3 | 33.3 | 48.1 | 38.4 | 48.6 | **46.7** | **NL** | *carnica* |
| Lab 10 | 35.7 | 32.3 | 36.1 | 33.9 | 21.2 | 46.1 | 39.3 | 32.7 | 34.2 | 40.5 | **35.2** | **CH** | *carnica* |
| Lab 11 | 25.3 | 32.1 | 38.1 | 40.0 | 25.5 | 42.8 | 23.9 | 40.4 | 22.5 | 45.4 | **33.6** | **UK** | *buckfast* |
| Lab 12 | 19.6 | 19.9 | 24.2 | 27.2 | 45.7 | 47.7 | 63.9 | 43.2 | 62.1 | 30.3 | **38.4** | **USA** | *ligustica* |
| Lab 13 | 41.2 | 37.0 | 46.4 | 34.3 | 37.2 | 29.5 | 38.3 | 47.4 | 25.8 | 50.9 | **38.8** | **D** | *carnica* |
| Lab 14 | 61.4 | 57.5 | 65.8 | 67.2 | 70.9 | 66.5 | 68.8 | 54.8 | 74.4 | 52.6 | **64.0** | **F** | *buckfast* |
| Lab 15 | 7.5 | 16.3 | 31.5 | 25.6 | 33.6 | 28.4 | 28.4 | 37.3 | 25.2 | 41.3 | **27.5** | **D** | *carnica* |
| Lab 16 | 23.0 | 20.3 | 33.8 | 33.9 | 19.4 | 38.0 | 25.9 | 25.8 | 22.6 | 36.7 | **27.9** | **D** | *carnica* |
| Lab 17 | 30.0 | 60.0 | 63.3 | 66.7 | 30.0 | 70.0 | 46.7 | 50.0 | 46.7 | 33.3 | **49.7** | **I** | *ligustica* |

D = Germany; E = Spain; USA = USA; F = France; Ch = Switzerland; GB= Great Britain;
I = Italy; NL = Netherlands

| | |
|---|---|
| mean | 40.8 |
| min.: | 27.5 |
| max.: | 64.0 |

BASF
The Chemical Company

**BASF SE**
APR/DP - Product Characterization & Performance Management

BASF SE - Crop Protection - Speyerer Strasse 2, D-67117 Limburgerhof, Germany

## Certificate of Analysis

| | | | |
|---|---|---|---|
| **BAS-No. :** | **BAS 152 11 I** | **Batch No. :** | **FRE-000926** |
| **Formulation Type :** | EC | **Date of Production :** | Mar 06, 2012 |
| **Date of Initial Analysis :** | Jul 15, 2013 | **Study Code :** | 711214_1 |

| Ingredient | | Nominal content | Analyzed content | Determination by |
|---|---|---|---|---|
| Name/Reg.No. | BAS Code | | | |
| Dimethoate | BAS 152 I | 400.0 g/L | 400.9 g/L | GC |

*The results are within the FAO tolerances for plant protection products. (Manual on development and use of FAO and WHO specifications for pesticides - November 2010 second revision of the First Edition, Rome, 2010).*

**Density :** 1.069 g/cm³

**Homogeneity :** given

***Additional Information***

**Storage Advice:** keep at room temperature (typically +25°C) or cooler

**Expiration Date:** Jun 17, 2015

**Remarks:** Omethoate 0.26 % ; Isodimethoate = 1.50 %

*Recipients should ensure that the label information on the corresponding substance container(s) correspond(s) with that on this Certificate of Analysis*

**Study Director :** Siebecker, Michael

**Study Completion Date :** August 06, 2013

**Issued on :** August 22, 2013

**Issued by :** H. Dukelie

*This document has been computer generated and is based on the GLP-studies cited above*
*Released August 06, 2013 by Siebecker, Michael*

FIGURE 5 Certificate of Analysis of Perfekthion (dimethoate)

**PART 2**

**REPORT –**
**SUMMARY OF THE RESULTS OF THE 2ND INTERNATIONAL RING TEST FOR THE STANDARDISATION OF A 10 DAY CHRONIC FEEDING TEST ON HONEY BEES (*APIS MELLIFERA* L.) IN THE LABORATORY**

# TABLES



# FIGURES

## INTRODUCTION

65. Pollinators like honey bees (*Apis mellifera*) may be exposed to residues of plant protection products (PPP) for a prolonged period of time, *e.g.* via ingestion of contaminated food, stored and consumed by the bees in the hive or by foraging nectar and pollen of (seed) treated crops. To assess this potential risk a chronic toxicity test can be conducted in the laboratory. Here, young adult worker bees (maximum 2 days old) are continuously exposed to treated food over a period of 10 days. This 10 day chronic feeding test should be used to cover a prolonged exposure phase and to determine endpoints like $LC_{50}$/$LDD_{50}$ (Lethal Concentration$_{50}$/Lethal Dietary Dose$_{50}$) and NOEC/NOEDD (No Observed Effect Concentration/No Observed Effect Dietary Dose), where possible.

66. In order to collect and compile practical experience already gained by the laboratories and to harmonize and standardize the testing procedure, two independent ring tests were conducted in 2014 and 2015. The results of the first ring test were presented and discussed during an expert meeting in Paris, April 22 -24, 2015. During this meeting it was agreed to set up another ring test round in 2015 in order to address open questions. The scope of this 2$^{nd}$ international ring test in 2015 was to assess possible side-effects of the solvent acetone at different concentrations and to investigate the occurrence and outcome of possible evaporation of the feeding solution. One additional test was conducted in one laboratory in order to assess the influence of the number of bees per test cage on the natural mortality of the bees during the 10 day test period. This test also investigated possible colony effects which might play a role when using bees of one versus several colonies for a test.

67. In total 16 laboratories from 9 countries including six bee institutes/universities, two industry laboratories and 8 contract laboratories participated in this ring test. On total 19 sets of data were provided by the participants.

68. Every participating laboratory followed the recommendations of the recent Test Guideline Proposal after revision by the international OECD Bee Expert Group (Paris, April 22 – 24, 2015).

69. In the 2015-ring test three concentrations of acetone were tested: The bees were continuously fed with 1, 3 and 5 % acetone, offered in 50 % sugar solution (solvent treatment group). One concentration of the reference item (a.i. dimethoate), was used in each test (reference item group). The concentration of the reference item was chosen by each lab individually, but should result in a mortality of ≥ 50 % at the end of the test. One untreated control group was included in the test (untreated 50 % w/v aqueous sugar solution).

70. Food loss due to evaporation was determined by placing three additional test cages, without bees but with feeders introduced (untreated sugar solution and sugar solution containing 5 % acetone) beside the actual test cages. Evaporation in these cages was assessed by daily weighing of the feeders. The weight assessments and exchange of the "evaporation"-feeders were conducted daily in the same way as for the actual test cages throughout the whole 10 day test period.

71. The following table gives an overview of the set-up of the 2$^{nd}$ international ring test:

**Overview and Details for Material and Methods**

| **Treatment Group** | **Concentration** | **No. of Rep./Test Units** |
|---|---|---|
| Untreated Control | Pure 50 % sugar solution | 3 |
| Acetone 1 | 1 % acetone in 50 % sugar solution | 3 |
| Acetone 2 | 3 % acetone in 50 % sugar solution | 3 |
| Acetone 3 | 5 % acetone in 50 % sugar solution | 3 |
| Reference Item | One concentration in50 % sugar solution | 3 |
| Evaporation Untreated Control | Pure 50 % sugar solution (without bees) | 3 |
| Evaporation solvent Control | 5 % acetone in 50 % sugar solution (without bees) | 3 |
| **Sum:** | | **21** |

72. The presented data and experiences confirmed the proposed validity criteria for the controls as well as the proposed procedure and rates for the reference item treatment. It also showed that the use of acetone up to a concentration of 5 % is possible. Evaporation occurred in all tests and it is suggested to determine its extent and if necessary to adjust the calculated food consumption per bee for the observed food loss. Evaporation depends on a number of technical reasons such as type of feeder, size of the access hole of the feeder, relative humidity, volatility of the solvent etc. Therefore, when detected and measured it might be technically reduced and/or included in the calculation of the food consumption of the bees in order to determine realistic and conservative data on food consumption and likewise to avoid an overestimation of the actual food consumption of the bees.

73. In all participating laboratories the validity criterion of ≤ 15 % was met for the untreated control group in all tests within the first run. For the solvent groups (50 % sugar solution containing 1, 3 or 5 % acetone, respectively) this validity criterion was met for all solvent treatments in 16 out of 18 tests. In two tests the validity criterion was not met for one of the three solvent treatments. Both incidents must not be overestimated since in one case the increased mortality can be considered as incidental (confirmed by a generally high number of valid contract studies conducted by this lab) and in the other case the test was performed late in the season (end of September).

74. The tests were conducted under non-GLP conditions; however, most of the participating labs were GLP certified.

75. In the present report, the results of the solvent and evaporation experiments reflect the data of labs 1 to 15 comprising 18 data sets. Lab 16 performed an independent experiment (effect of the number of bees per cage and possible colony effects) and was not included in the evaluation of the solvent and evaporation issue.

**INFORMATION ON THE RING TEST GROUP**

In total 16 laboratories from 9 countries participated in the ring test group:

| Laboratory | Responsible person | Address and contact details |
|---|---|---|
| Lab 1 | Stephan Schmitzer | Ibacon GmbH, Arheilger Weg 17, D-64380 Rossdorf, Germany<br>Phone: +49 6154 697-376,<br>Email: stephan.schmitzer@ibacon.com |
| Lab 2 | Annette Kling | Eurofins Agroscience Services Ecotox GmbH<br>Eutinger Str. 24<br>75223 Niefern-Öschelbronn, Germany<br>Phone: +49 (0)7233 - 9627 465;<br>Email: AnnetteKling@eurofins.com |
| Lab 3 | Amandine Vincent | Ecotox Services Australasia Pty Ltd, and<br>Ecotox Services International<br>27 / 2 Chaplin Drivea<br>Lane Cove NSW 2066 Australia<br>Email: avincent@ecotox.com.au |
| Lab 4 | Olga Gálvez | Agricultura y Ensayo S.L.<br>Urb. El Eucaliptal C/Cordialidad, 9. 41500 Alcalá de Guadaira Seville, Spain<br>Email: ogalvez@agriculturayensayo.com |
| Lab 5 | Claudia Volles | Agroscope, Zentrum für Bienenforschung, Schwarzenburgstrasse 161,<br>3003 Bern, Switzerland<br>Email: claudia.volles@agroscope.admin.ch |
| Lab 6 | Dagmar Sack<br>Stefanie Niederdrenk | BASF SE, D-67056 Ludwigshafen, Germany<br>Email: dagmar.sack@basf.com |
| Lab 7 | David Gladbach | Bayer CropScience AG, 40789 Monheim, Germany<br>Email: david.gladbach@bayer.com |
| Lab 8 | Sophie Krainer | Karl-Franzens-Universität Graz<br>Institut für Zoologie<br>Universitätsplatz 2<br>8010 Graz, Austria<br>Email: sophie.krainer@uni-graz.at |
| Lab 9 | Julie Furrier | ACTA<br>ICB – VetAgroSup - 1 avenue Claude Bourgelat, 69280 MARCY L'ETOILE, FRANCE<br>Email: julie.fourrier@acta.asso.fr |
| Lab 10 | Saskia Ruhland | BioChem agrar GmbH<br>Kupferstraße 6<br>04827 Machern / OT Gerichshain, Germany<br>Email: Saskia.Ruhland@biochemagrar.de |
| Lab 11 | Emma Wright<br>Selwyn Wilkins | Centre for Chemical Safety and Stewardship<br>The Food and Environment Research Agency<br>Sand Hutton, York YO41 1LZ, UK<br>Email: selwyn.wilkins@fera.gsi.gov.uk |

| | | |
|---|---|---|
| Lab 12 | Claire Molitor | Testapi SARL, 464, Sarré, 49350 Gennes, France<br>Email: molitor.testapi@orange.fr |
| Lab 13 | Dorothee Lüken | Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES), Institut für Bienenkunde Celle, 29221 Celle, Germany.<br>Email: Dorothee.Lueken@LAVES.Niedersachsen.de |
| Lab 14 | Monica Colli | Biotecnologie BT S.r.l., C.F. e P. IVA 02384950545<br>Fraz. Pantalla 06059 Todi (PG), Italy<br>Email: mcolli@biotecnologiebt.it |
| Lab 15 | Ming Hua Huang | Eurofins Agroscience Services, Inc.,15052 NC Highway 86 S, Prospect Hill, NC 27314, USA<br>Email: minghuahuang@eurofins.com |
| Lab 16** | Pierrick Aupinel | INRA<br>147 rue de l université 75338 paris cedex 07, France<br>Email: Pierrick.Aupinel@magneraud.inra.fr |

Cells marked in grey indicating the leaders of the ring test group

**Provided data on the possible influence of the number of bees per test cage and possible colony effects

Further consulting member of the ring test group:

Jens Pistorius Julius Kühn-Institut (JKI) Bundesforschungsinstitut für Kulturpflanzen Messeweg 11-12, 38104 Braunschweig. E-Mail: jens.pistorius@jki.bund.de

**TIME SCHEDULE**

| **Activity** | **Scheduled date** |
|---|---|
| 1st international Ring Test Meeting | May 08, 2014 |
| 1st international Ring Test: Experimental Start | May 2014 |
| 1st international Ring Test: Experimental Completion | July 2014 |
| Delivery of Report and GL Proposal to OECD | December 18, 2014 |
| OECD Expert Meeting, Paris | April 22-24, 2015 |
| 2nd international Ring Test: Experimental Start | May 2015 |
| 2nd international Ring Test: Experimental Completion | September 2015 |

## MATERIALS AND METHODS

### Solvent, Reference Item and Control Groups

*Solvent*

76. Acetone with a high purity (p.a. quality, purity ≥ 99 %) was used to prepare the feeding solutions with different concentrations of acetone. The quality and source was provided by the participating labs. The following concentrations of acetone in the feeding solutions were used: 1, 3 and 5 % acetone in 50 % w/v aqueous sugar solution.

*Reference Item*

77. The active ingredient dimethoate was used as a reference item at one concentration. Either a formulation (*e.g.* BAS 152 11 I) or the technical active ingredient was used in the test. The concentration of the reference item was chosen by each lab individually, but should result in a mortality of ≥ 50 % at the end of the test. The range of concentrations tested was 0.6 to 1.16 mg dimethoate/kg feeding solution among the performing laboratories.

*Controls*

78. In the untreated control group, the bees received untreated 50 % (w/v) aqueous sugar solution *ad libitum.* The sugar solution was prepared with deionized water. In order to investigate possible evaporation from the feeding solutions, two additional "Evaporation Control Groups" were included in the ring test. These additional test cages did not contain bees, but feeders filled with:

a) untreated 50 % (w/v) aqueous sugar solution (3 replicates;(cages));

b) 50 % (w/v) aqueous sugar solution containing 5 % acetone (3 replicates;(cages))

**Test Organism / Test System**

| | |
|---|---|
| Species/Variety | *Apis mellifera* L. (Hymenoptera, Apidae) |
| Life stage | Young adult worker bees (maximum 2 days old) |
| Source | Commercial supplier or own stock beehives. The source, including the name and address of the beekeeper, are documented in the ring test raw data. |
| Stock keeping | The hive(s) used for honeybee collection for this ring test were adequately fed, healthy and as far as possible disease-free and queen-right with known history and physiological status.<br>No chemical substances (such as antibiotics, anti-*Varroa* treatments, pesticides, *etc.*) were used in the hives for at least one month prior to this test.<br>Colony inspections and stock keeping of honeybees were recorded by the respective laboratories. |
| Collection of the bees | Brood combs with sealed brood from one or more hives were used to obtain the number of bees needed for the test. If several combs were used, it was suggested that the combs were combined during the hatching of the bees to ensure that the bees mixed naturally. |
| Hatching period | Two methods of collection of the bees were provided:<br><br><u>Method 1: Hatching in an incubator</u><br>One day prior to test start, one or more brood comb(s) were taken out of a honeybee colony and transferred into a climatic chamber or incubator. Adult honey bees sitting on these combs were brushed off previously. The combs were kept under test conditions (33 ± 2 °C) for one day. In case the brood comb(s) did not contain enough honey and pollen as food for the hatched bees, an additional pollen and/or honey comb could have been placed beside the brood combs (This method was used by most of the labs).<br><br><u>Method 2: Hatching inside the hive</u><br>One day prior to test start, one or more brood comb(s) with sealed brood from one hive were used in the test in which bees were visibly starting to emerge. These combs contained pollen which was used as a first feeding source for the freshly hatched bees. The combs were taken out from the hive and adult bees were swept off. Each comb was placed in an excluder box and was returned to the hive for one further day. The freshly hatched bees remained in the excluder box in the hive and were fed by nurse bees until they were collected for the test. |
| Acclimatisation period | After a hatching period of one day, the bees were taken from the hatching box in the incubator or the excluder box from the hive and transferred to test units. The bees were then acclimatized under test conditions for one further day.<br>No additional feeding of pollen and water was supplied during acclimatization and test period.<br>Dead and moribund bees were rejected and replaced by healthy bees before test start.<br>Care was taken by using suitable tools (*e.g.* forceps) to avoid damaging of the bees during the collection. |

**Test Design**

| | |
|---|---|
| Exposure time | 10 days |
| Number of treatment groups | 1 control group (untreated control), 3 concentrations of the solvent acetone (1, 3 and 5 %), 2 evaporation controls (untreated and 5 % acetone control), 1 reference item group (dimethoate). |
| Replicates per treatment group | 3 |
| Honey bees per treatment group | 30, divided into 3 replicates, each containing 10 bees per cage. This number allows a precise quantitative assessment of affected *vs.* non-affected bees. Exception: lab 12 tested 14 bees/replicate in the untreated control treatment)<br>For the evaporation group, no bees were used, only the bare test units with feeders contain either untreated 50 % w/v sugar solution or sugar solution containing 5 % acetone. |

**Test Cages**

| | |
|---|---|
| Test cages | The cages used were well-ventilated and made of material which was either easy to clean (reusable) or disposable.<br>Most of the labs used reusable test cages made of stainless steel.<br>The size of the cages was appropriate to the number of bees *i.e.* providing adequate space.<br><br>Example for a steel cage:<br>The front side of the cage is equipped with a transparent pane to enable observation. The bottom of the cage consists of perforated steel, which guarantees sufficient air supply. The cages were lined with filter paper. |

**Test Conditions**

| | |
|---|---|
| Temperature | 33 ± 2°C |
| Relative humidity | 50 - 70 % (humidity range in most of the labs were broader than the targeted values) |
| Exposure to light | Constant darkness except during the exchange of feeders and assessments |
| Ventilation | The test room/climatic chambers were ventilated. |
| Feeding | During acclimatisation: 50 % (w/v) aqueous sugar solution *ad libitum.*<br>During test period: continuously *ad libitum* with treated or untreated feeding solutions |
| Specific documentation | Temperature and humidity were recorded continuously with appropriate, calibrated equipment.<br>(Short-term deviations (< 2 hours) from the recommended temperature and humidity ranges are unavoidable, should not have affected the integrity or outcome of the study and were not considered as deviations). |

**Feeding Solutions**

| | |
|---|---|
| Feeding solutions | The feeding solutions for the control and acetone treatments were prepared with 50 % (w/v) sugar solution. |
| Specific documentation | The bees were fed continuously *ad libitum* with a 50 % (w/v) sugar solution containing either:<br>• acetone at 3 different concentrations (solvent treatment group),<br>• pure 50 % (w/v) sugar solution (untreated control group),<br>• reference item containing dimethoate (reference item group)<br>The treated and untreated food was offered using plastic syringes or Eppendorf tubes as feeders. Every day the feeders were replaced by new ones filled with fresh treated or untreated food. |

**Application**

***Preparation of the Control and Reference Item Feeding Solutions***

| | |
|---|---|
| Preparation of the control feeding solution | The feeding solution for the untreated control group (and untreated evaporation group) was 50 % (w/v) aqueous sugar solution.<br>The untreated control feeding solutions were prepared at least every 4 days and stored in the refrigerator at 6 °C ± 2 °C. |
| Preparation of the solvent (acetone) treatment group | The feeding solutions for the solvent (acetone) treatment groups (and solvent evaporation group) were prepared with 50 % (w/v) aqueous sugar solution and high quality acetone. These solutions contained 1, 3 or 5 % acetone.<br>The solvent treatment group feeding solutions were prepared at least every 4 days and were stored in the refrigerator at 6 °C ± 2 °C. |
| Preparation of the reference item feeding solution(s) | A stock solution of the reference item was prepared once for the whole test period and stored in the refrigerator at 6°C ± 2 °C for up to 10 days. Deionised water was used as solvent.<br>The reference item feeding solutions were prepared from the stock solution at least every 4 days and were stored in the refrigerator at 6°C ± 2°C.<br>[dimethoate is stable in deionised water and in sugar solution over a period of 10 days when stored in the refrigerator at 4 °C ± 4 °C; Internal BASF SE analytical report (March 25, 2014), IBACON analytical report (79371136A, March 19, 2014)] and Eurofins Agroscience Services Ecotox GmbH analytical results (May 19, 2015). |
| Preparation of the evaporation feeding solutions | For the evaporation measurements the same feeding solutions as for the definitive test with honey bees were used. Preparation mentioned above. |

***Application Details***

| | |
|---|---|
| Concentrations of acetone | 1, 3 and 5 % acetone |
| Concentration of the reference item: | One concentration of dimethoate in the feeding solution was tested. Each lab chose its own concentration level which ranged from 0.6 to 1.16 ppm. |
| Application mode | The treated and untreated feeding solutions were offered to the test organisms via feeders introduced into each test unit (*e.g.* plastic syringes, approx. 10 mL or Eppendorf tubes). If syringes were used as feeders, the tip of each syringe was removed so that the bees had access to the feeding solution.<br>In parallel to the application of treated and untreated food, test cages without bees but with untreated and 5 % acetone treated sugar solutions were used to measure evaporation. These test units did not contain bees. |
| Amount of feeding solution | The quantity of food offered to each cage should have exceeded the amount actually needed by the bees in order to guarantee an *ad libitum* feeding. The bees in one test unit shared the feeding solution and thus received similar doses (trophallaxis). |

| | |
|---|---|
| Application procedure | Every day (± 2 h in relation to the 1st application) the feeders containing the untreated control, acetone and reference item feeding solutions were replaced by fresh ones (one application interval). The treated and untreated feeding solutions were offered *ad libitum* to each cage in feeders. The feeders were weighed daily before introduction into the cages and after the feeding interval. |
| Dose verification | The amount of feeding solution(s) consumed was determined by weighing the feeders before and after feeding, using calibrated equipment. |

***Time Schedule***

| **Time in Relation to the first Application** | **Action** |
|---|---|
| Day - 2 to day -1 | Incubation of the brood combs |
| Day - 1 to day 0 | Collection of bees and acclimatisation |
| Day 0 = 1st Application | Preparation of stock solution(s), preparation of feeding solution(s), weighing of feeders; 1st application |
| Day 1 to day 9 (± 2 h in relation to 1st Application) | Assessment of mortality and behavioural abnormalities; preparation of feeding solutions (at least every four days), daily replacement of feeding solutions by fresh ones, determination of consumed feeding solutions and evaporation by weighing the feeders. |
| Day 10 (± 2 h in relation to 1st Application) | Assessment of mortality and behavioural abnormalities; determination of consumed feeding solutions or determination of weight loss by evaporation by weighing the feeders |

**Assessments**

***Mortality and Food Consumption***

| | |
|---|---|
| Mortality | Mortality was assessed and recorded daily at about the same time of the day (every 24 h ± 2h), for a period of 10 days starting 24 h ± 2 hours after start of the test period until test end. |
| Food consumption | The food consumption per bee was calculated by the number of living bees at start of each feeding interval and the amount of food taken up until the following day.<br>Food consumption was assessed and recorded daily at about the same time of the day (every 24 h ± 2h) for a period of 10 days and, starting 24 ± 2 hours after start of the test period.<br>Food consumption was calculated for two scenarios:<br>• without evaporation (uncorrected uptake)<br>• corrected for evaporation (corrected uptake). |
| Evaporation: | Evaporation for the untreated control and the 5 % acetone treatment group was assessed. For the 1 and 3 % acetone solvent treatment groups the evaporation data of the untreated control were used to calculate the corrected food uptake. |

Evaporation was assessed and recorded daily in parallel to the assessment of the food consumption assessment (every 24 h ± 2 h) for a period of 10 days and, starting 24 ± 2 hours after start of the test period

## DATA EVALUATION

### *Evaluation of Mortality*

79. Results obtained from the bees treated with the solvent treatment group were compared to those obtained from the control fed with untreated sugar solution. The percentage of mortality was calculated for each treatment group from the number of dead individuals in correlation to the number of introduced test organisms. The data of the reference item and solvent treatment group were not corrected for control mortality.

### *Evaluation of Food Consumption and Evaporation*

*Food Consumption (without correction for evaporation)*

80. The weight of the feeders was recorded before and after feeding to the bees in order to calculate the exact food consumption per bee per day.

81. The difference in the weight before and after feeding the bees represents the food consumed by the bees in one cage during one feeding interval (about 24 hours) (= uncorrected food uptake). This amount of food was divided by the number of living bees at the start of the corresponding feeding interval (*e.g.* day 4 – 5: consumed food at day 5 divided by the living bees at day 4).

82. The mean food consumption per bee was calculated from the three replicates per treatment and day and also over the whole test period.

83. For the reference item treatment the mean uptake of reference item is presented for each day as well as accumulated over the entire test period. The mean uptake of reference item per bee per day over the whole testing period was calculated by averaging the single replicate values over the relevant test days (on which bees were alive).

*Food Consumption (corrected for evaporation)*

84. The weight of the feeders of the additional evaporation cages was recorded before and after feeding to the bees in order to calculate the exact evaporation values [mg/test cage]. The weight loss of the feeding solution due to evaporation was subtracted from the calculated uptake to give the corrected uptake. For the untreated control group as well as for the 1 % and 3 % acetone treatment group the values of the untreated evaporation group was used. For the 5 % acetone treatment group the values from the 5 % acetone evaporation group were used. The corrections were performed on a daily basis.

85. The difference in the weight before and after feeding the bees, including the loss due to evaporation represents the food consumed by the bees in one cage during one feeding interval (ca. 24 hours) (= corrected food uptake). This amount of food was divided by the number of living bees at the

start of the corresponding feeding interval (*e.g.* day 4 – 5: consumed food at day 5 divided by the living bees at day 4).

The mean food consumption per bee, corrected for evaporation was calculated from the three replicates per treatment per day and as a mean over the whole test period.

86. For the reference item treatment, the mean corrected uptake of reference item is presented for each day as well as accumulated over the entire test period. The mean uptake of reference item per bee per day over the whole testing period was calculated by averaging the single replicate values over the relevant test days (on which bees were alive).

87. In cases where the evaporation values were higher than the weight difference in the actual test cages and correction for evaporation would have led to negative food consumption, the corrected food consumption was set to "0 mg/bee/day".

*Evaporation*

88. The "evaporation"-feeders containing the untreated and 5 % acetone treated feeding solutions were weighed and replaced daily in parallel to each feeding interval of the bees (daily)..

89. The difference between the weight before and after one time interval represents the weight loss of the respective feeding solution by evaporation in one cage during 24 hours. The evaporation figure was then subtracted from the calculated uptake to give the corrected uptake considering the loss by evaporation. The mean weight loss of solution per test cage *via* evaporation is presented as an average value for each day as well as for the entire test period.

***Statistics***

90. Apart from the experiments with solvent and evaporation an additional data set was generated to investigate possible effects of the number of bees per cage on the mortality. This data set was statistically evaluated. The data on the number of bees per cage were normally distributed but not homogenous, therefore Welch t-test, one-sided smaller, pairwise comparison, one-sided greater, $\alpha = 0.05$, was used.

91. For the data set on the effect on colony level on the outcome of mortality, Kruskal-Wallis-test Procedure, and an ANOVA, $\alpha = 0.05$ was used. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

## VALIDITY CRITERION

| | |
|---|---|
| Control mortality | The validity threshold for mean mortality in the untreated control group as well in the solvent treatment groups at the end of the test was set to ≤ 15 %.<br>This validity criterion was adopted from the validity criterion of the EPPO 170 guideline (≤ 15 %) and OECD TG 213 (≤ 10 %), by taking into consideration the prolonged test period of 10 days. |

## DEVIATIONS TO THE PROTOCOL

- one lab (lab 12) used 14 bees per cage in the untreated control group.
- one lab (lab 7) provided two data sets with an untreated control group and a 5 % acetone treatment group only (7a and 7b) (*i.e.* no 1 % and 3 % acetone treatment groups and no reference item data)
- one lab (lab 5) provided 2 data sets; one was complete and with syringes as feeders (5a) and one data set (5b) comprised 3 % and 5 % acetone treatment groups with tubes as feeders.
- lab 2 provided 2 data sets

## RESULTS AND DISCUSSION

### Mortality Results of the Reference Item

TABLE 1 and FIGURE 6 present the cumulative mortality in the reference item group over the 10- day test period.

**TABLE 28 Cumulative mortality [%] in the reference item treatment group during the 10 days test period**

| | | Cumulative mortality [%] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lab** | **Concentration [mg a.i./kg]** | **Day 1** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | **Day 6** | **Day 7** | **Day 8** | **Day 9** | **Day 10** |
| 1 | 1.0 | 0.0 | 0.0 | 10.0 | 53.3 | 80.0 | 100 | 100 | 100 | 100 | 100 |
| 2a | 0.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10.0 | 80.0 | 96.7 | 100 | 100 |
| 2b | 0.9 | 0.0 | 0.0 | 0.0 | 16.7 | 20.0 | 20.0 | 46.7 | 83.3 | 96.7 | 100 |
| 3 | 0.9 | 0.0 | 0.0 | 10.0 | 23.3 | 80.0 | 93.3 | 96.7 | 100 | 100 | 100 |
| 4 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 6.7 | 43.3 | 70.0 | 90.0 | 100 |
| 5a | 1.0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 6 | 1.0 | 0.0 | 0.0 | 13.3 | 53.3 | 63.3 | 96.7 | 96.7 | 100 | 100 | 100 |
| 8 | 0.9 | 0.0 | 3.3 | 16.7 | 40.0 | 76.7 | 100 | 100 | 100 | 100 | 100 |
| 9 | 0.8 | 0.0 | 3.3 | 3.3 | 6.7 | 13.3 | 50.0 | 100 | 100 | 100 | 100 |
| 10 | 0.7 | 0.0 | 0.0 | 0.0 | 3.3 | 6.7 | 13.3 | 33.3 | 56.7 | 90.0 | 100 |
| 11 | 1.0 | 0.0 | 0.0 | 36.7 | 80.0 | 100 | 100 | 100 | 100 | 100 | 100 |
| 12 | 1.16 | 0.0 | 0.0 | 0.0 | 10.0 | 40.0 | 70.0 | 86.7 | 93.3 | 96.7 | 100 |
| 13 | 0.9 | 0.0 | 0.0 | 3.3 | 60.0 | 93.3 | 96.7 | 100 | 100 | 100 | 100 |
| 14 | 0.7 | 0.0 | 0.0 | 0.0 | 13.3 | 13.3 | 23.3 | 50.0 | 56.7 | 73.3 | 83.3 |
| 15 | 0.9 | 0.0 | 0.0 | 0.0 | 36.7 | 70.0 | 96.7 | 96.7 | 100 | 100 | 100 |
| **Mean** | **0.88** | | | | | | | | | | **98.9** |

Remark: The data set of lab 5b, 7a and 7b did not include reference item data

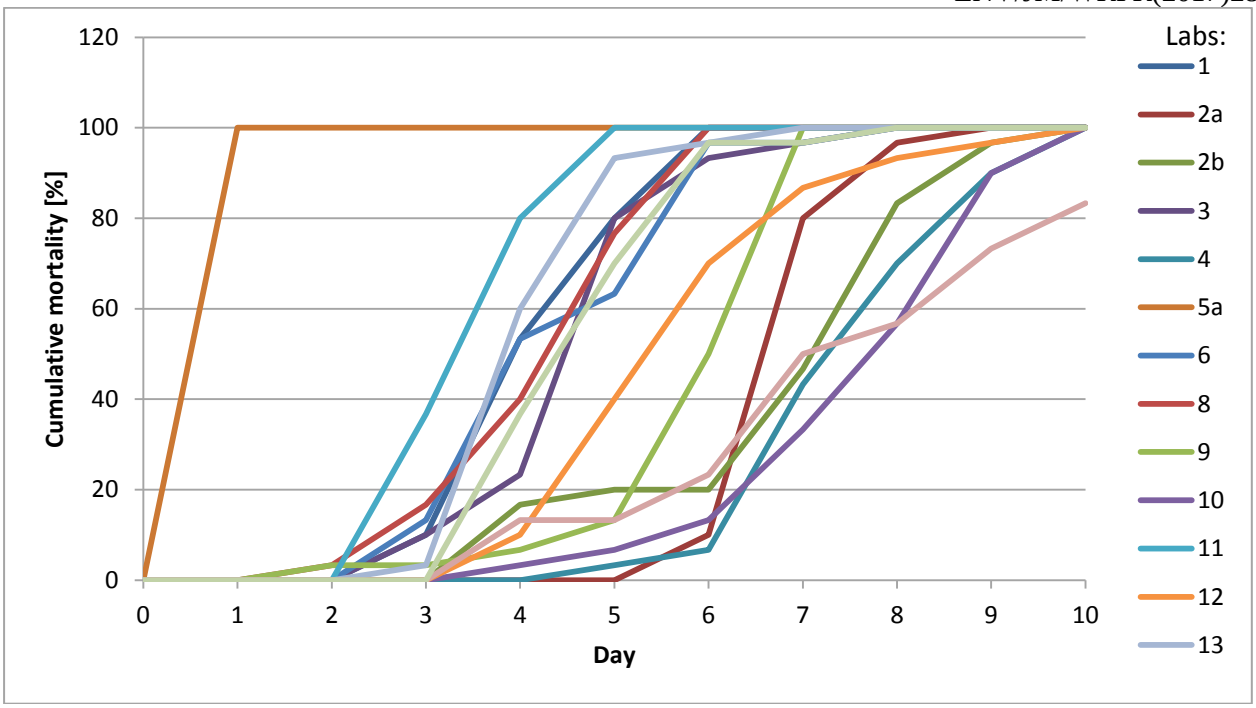


**FIGURE 6 Cumulative Mortality [%] in the reference item treatment group over 10 days**

92. A number of 15 data sets including data on the reference item were provided by the participating labs. The concentration range of the reference item (dimethoate) treatment between the labs was 0.6 to 1.16 mg dimethoate/kg feeding solution. In all labs the mortality continuously increased with the duration of exposure. The final mortality at the end of the 10 day exposure period was 100 % in all labs (exception: lab 14 with 83.3 % mortality) following a chronic exposure.

**Mortality Results of the Untreated Control Group**

93. The results of the cumulative mortality in the untreated control group are presented in the following table:

**TABLE 29 Cumulative mortality [%] in the untreated control group during the 10 days test period**

| Untreated control*: Cumulative mortality [%] | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lab 1** | **Lab 2a** | **Lab 2b** | **Lab 3** | **Lab 4** | **Lab 5a** | **Lab 5b**** | **Lab 6** | **Lab 7a** | **Lab 7b** | **Lab 8** | **Lab 9** | **Lab 10** | **Lab 11** | **Lab 12***** | **Lab 13** | **Lab 14** | **Lab 15** | **Mean** |
| 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 0.0 | - | 10.0 | 3.3 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 2.4 | 0.0 | 6.7 | 0.0 | **3.1** |

* untreated control group was fed with 50 % aqueous sugar solution
** in this data set of lab5b only data on 3 and 5 % acetone were provided
*** 14 bees were used per cage

One control group fed with untreated 50 % w/v sugar control was included in the ring test.

94. For the untreated control, all labs met the internal validity criteria of ≤ 15 % within the first run. Therefore all 16 tests (performed by 15 labs) were deemed valid. Mortality levels for the untreated control fed with pure 50 % w/v sugar solution ranged from 0.0 % to 10.0 %, resulting in a mean mortality level over all labs of 3.1 %.

**Mortality Results of the Acetone (Solvent) Treatment Groups**

95. Since many test items (mainly technical substances) are of low water solubility it is of paramount importance to have a suitable solvent available for this kind of test. As most of the common solvents are proved to have an intrinsic mortality (unpublished data, Niederdrenk *et al*., 2015), acetone recently seems to be the only choice for the time being.

96. However, in the first international ring test (2014), the mortality observed in the 5 % acetone solvent control group showed a great variability over the labs. In this 1st ring test, the mortality levels ranged from 0.0 % to 90.0 %, resulting in a mean value over the labs of 18.8 %. In more than half of the tests (10 out of 17) the validity criterion of ≤ 15% was met also for the 5 % acetone solvent control group. But in 7 labs the mortality was beyond the accepted control mortality.

97. After detailed investigation of the data from 2014 we came to the conclusion that major parameters like *e.g.* bee race, acetone quality, age of bees, country of test conduct are not linked to the increased solvent control mortality.

98. In the first ring test from 2014 it was observed that in the respective labs the mortality did not continuously increase but started to rise mainly from day 6 onwards. The observations led to the assumption that there could be a certain threshold for the testing of acetone in some labs. This would mean that the bees are able to metabolize the acetone up to a specific level, but are affected or die as soon as this level has been exceeded.

99. In order to prove or disprove this assumption it was decided to start another ring test in 2015, again with a 5 % acetone treatment but also with two lower concentrations (3 and 1 % acetone) The testing of 3 different concentrations should help indicating a possible dose-response correlation or to find out a possible mortality threshold to the bees (see TABLE 30 and FIGURE 7).

100. The results of the cumulative mortality in the different acetone solvent treatment groups are presented in the following table:

**TABLE 30 Cumulative mortality [%] in the 1, 3 and 5 % acetone group during the 10 days test period**

| Treatment* | Cumulative mortality after 10 days [%] | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Lab 1** | **Lab 2a** | **Lab 2b** | **Lab 3** | **Lab 4** | **Lab 5a** | **Lab 5b** | **Lab 6** | **Lab 7a** | **Lab 7b** | **Lab 8** | **Lab 9** | **Lab 10** | **Lab 11** | **Lab 12** | **Lab 13** | **Lab 14** | **Lab 15** | **Mean** |
| 0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 0.0 | - | 10.0 | 3.3 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 2.4 | 0.0 | 6.7 | 0.0 | **3.1** |
| 1 | 6.7 | 0.0 | 0.0 | 0.0 | 10.0 | 0.0 | - | 6.7 | - | - | 0.0 | 6.7 | 0.0 | 3.3 | 0.0 | 0.0 | 3.3 | 3.3 | **2.7** |
| 3 | 3.3 | 20.0 | 0.0 | 3.3 | 0.0 | 6.7 | 3.3 | 3.3 | - | - | 0.0 | 10.0 | 0.0 | 0.0 | 3.3 | 0.0 | 13.3 | 0.0 | **4.2** |
| 5 | 3.3 | 3.3 | 3.3 | 3.3 | 0.0 | 13.3 | 13.3 | 3.3 | 0.0 | 0.0 | 0.0 | 6.7 | 0.0 | 0.0 | 10.0 | 6.7 | 23.3 | 0.0 | **5.0** |

* % acetone in 50 % aqueous sugar solution

Remark: Cells marked in grey show mortalities beyond the validity criterion of ≤ 15 %

101. Seventeen out of the 18 tests showed that the highest tested acetone concentration of 5 % acetone/sugar solution caused mortality levels between 0 and 13.3 % after 10 days which was within the accepted range set for control mortality. Only in one lab (lab 14) an increased mortality of 23.2 % was observed at the end of the 10 day test period. This test was performed late in the season (end of September) which might explain the higher mortality level.

102. Similar observations were made at the lower concentration levels of 1 and 3 % acetone: a maximum mortality of 10 % was recorded in these treatments up to the final assessment after 10 days, with the exception of one data set (lab 2: 20 % mortality at 3 % acetone). Lab 2 repeated the test resulting in data which were in line with the other tests and historical results generated by this lab.

103. Conclusively it can be stated, that the effect of 5 % acetone which had occurred in some tests during the 1st ring test (showing an increased mortality) could not be confirmed in this 2nd ring test. Therefore acetone up to a concentration level of 5 % in the final feeding solution can be used in 10 day chronic feeding tests with honey bees (see TABLE 30 and FIGURE 7).

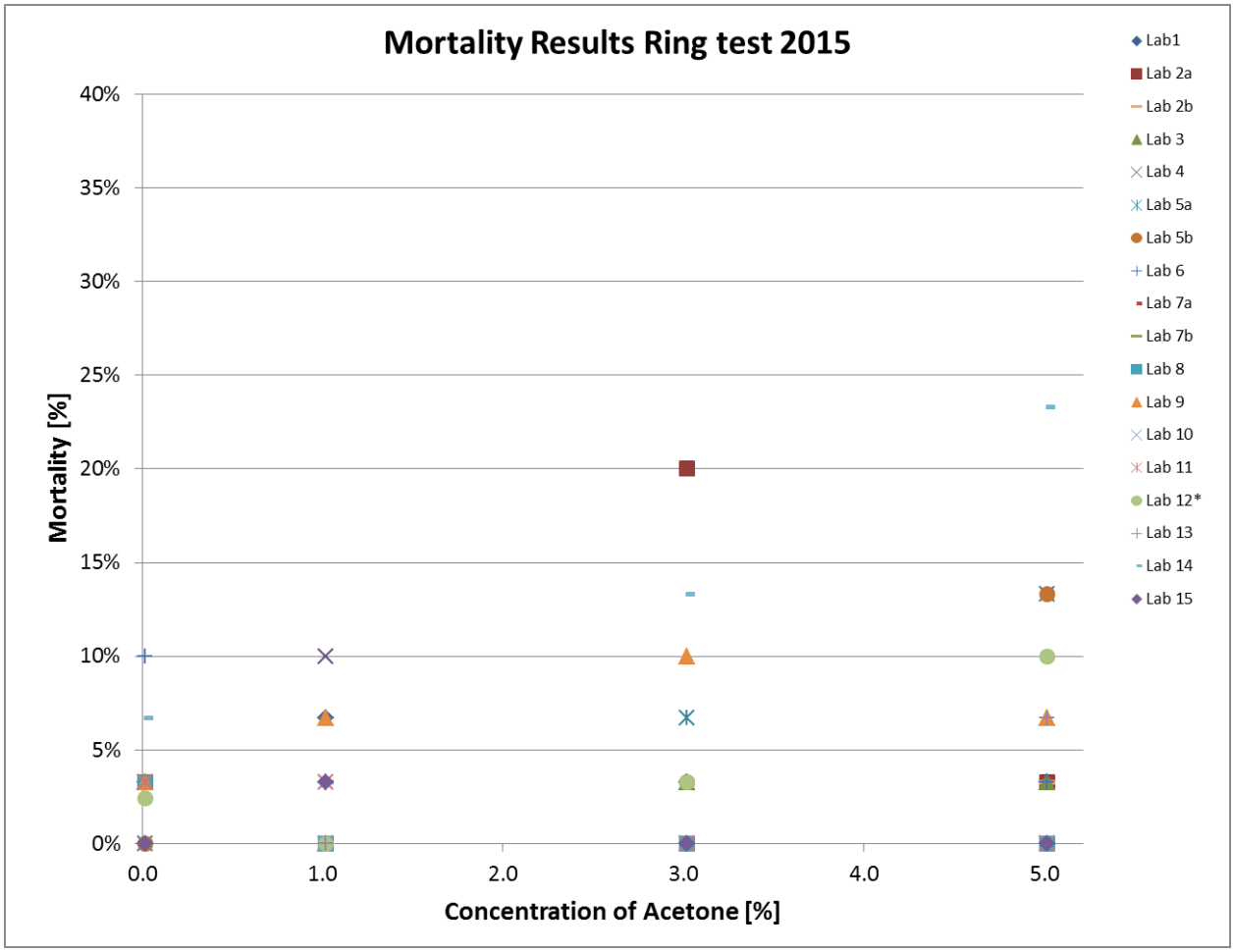


**FIGURE 7 Mortality [%] in the solvent treatment group over 10 days, treated with 50 % aqueous sugar solution containing 1, 3 and 5 % acetone**

**Details on Bees, Feeders and Acetone**

More details on the materials and methods of the tests performed are presented in the following table.

**TABLE 31 Details on the race of bees, age of bees, feeders, acetone purity**

| Lab 1 | Lab 2a | Lab 2b | Lab 3 | Lab 4 | Lab 5a | Lab 5b | Lab 6 | Lab 7a | Lab 7b | Lab 8 | Lab 9 | Lab 10 | Lab 11 | Lab 12 | Lab 13 | Lab 14 | Lab 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bee race** | | | | | | | | | | | | | | | | | |
| ca | ca | ca | n.a. | n.a. | n.a. | n.a. | ca | n.a. | n.a. | ca. | b | b | b | b | ca | li | li |
| **Bee age at test start [d]** | | | | | | | | | | | | | | | | | |
| 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| **Kind of feeder** | | | | | | | | | | | | | | | | | |
| S | S | S | S | T | S | T | S | S | S | S | T | S | T | S | S | S | S |
| **Purity Acetone** | | | | | | | | | | | | | | | | | |
| 99.5 | 99.7 | 99.7 | 99.8 | 99.5 | 99.8 | 99.8 | 99.5 | 99.5 | 99.5 | 99.5 | 99.8 | 99.8 | 99.9 | 99.9 | 99.7 | 99.0 | n.a. |

b = *Apis mellifera* buckfast
ca = *Apis mellifera* carnica
li = *Apis mellifera* ligustica
S = Syringes
T = Eppendorf Tubes
n.a. not applicable

**Food Consumption and Evaporation during the 10 Days Test Period**

104. The results of the food consumption, evaporation and the food consumption after correction of evaporation are presented in the following tables and figures.

105. Only those data sets were used where evaporation was measured.

106. The mean food uptake by the bees per day per bee over the 15 labs (16 data sets) without taking into consideration the evaporation was 37.7 mg untreated sugar solution per bee per day (minimum: 30.5 mg/bee/day; maximum 54.0 mg/bee/day). Mean uptake of feeding solution of the bees over the 16 data sets for the 1, 3 and 5 % acetone treatment groups were 36.2, 36.3 and 35.4 mg/bee day, respectively and thus comparable (see TABLE 32 and TABLE 33, Appendix TABLE 10 to TABLE 48). Based on the present results and the results of the previous ring test it can be concluded that no anti-feeding or repellent effect was caused by acetone which underlines the suitability of acetone as a solvent up to a concentration of 5 %.

107. Evaporation was measured in parallel to the untreated control group (50 % sugar solution) and in parallel to the 5 % acetone in 50 % sugar solution treatment group, respectively.

**TABLE 32 Mean consumption of feeding solution with and without consideration of evaporation in the untreated control group**

| Mean Consumption of the Bees and Evaporation: Untreated Control | | | | | | |
|---|---|---|---|---|---|---|
| **Lab** | **Mean uptake per bee without evaporation** | **Mean evaporation per cage** | **Mean uptake per bee with evaporation** | **Mean evaporation per living bee** | **Difference** | |
| | **[mg/bee]** | **[mg/cage]** | **[mg/bee]** | **[mg/bee]** | **[%]** | **Type of feeder** |
| **1** | 30.5 | 42.6 | 26.2 | 4.3 | 14.0 | S |
| **2a** | 32.7 | 70.1 | 25.7 | 7.0 | 21.4 | S |
| **2b** | 44.2 | 76.3 | 36.4 | 7.8 | 17.6 | S |
| **3** | 54.0 | 64.7 | 47.5 | 6.5 | 12.0 | S |
| **4** | 39.0 | 111.7 | 27.7 | 11.3 | 28.9 | T |
| **5** | 37.7 | 113.5 | 26.3 | 11.4 | 30.2 | S |
| **6** | 31.7 | 43.5 | 27.1 | 4.6 | 14.6 | S |
| **7** | 41.6 | 98.8 | 31.6 | 10.1 | 24.2 | S |
| **8** | 33.2 | 98.5 | 23.2 | 10.0 | 30.2 | S |
| **9** | 34.7 | 65.1 | 28.2 | 6.5 | 18.8 | T |
| **10** | 32.4 | 64.6 | 25.9 | 6.5 | 19.9 | S |
| **11** | 36.6 | 46.7 | 31.7 | 4.8 | 13.1 | T |
| **12** | 38.7 | 52.3 | 34.9 | 3.8 | 9.8 | S |
| **13** | 35.7 | 101.5 | 25.5 | 10.1 | 28.4 | S |
| **14** | 43.8 | 81.5 | 30.2 | 13.6 | 29.1 | S |
| **15** | 36.5 | 73.2 | 29.2 | 7.3 | 21.2 | T |
| mean | **37.7** | **75.3** | **29.8** | **7.8** | **20.8** | |
| minimum | 30.5 | 42.6 | 23.2 | 3.8 | 9.8 | |
| maximum | 54.0 | 113.5 | 47.5 | 13.6 | 30.2 | |

S = Syringes, T = Tubes *e.g.* Eppendorf Tubes

108. Weight loss of the untreated feeding solution without bees due to evaporation occurred in all labs ranging from 42.6 to 113.5 mg per day (mean: 75.3 mg/day) (see Table 5).

109. The average weight loss per day by evaporation (75.3 mg/day/cage) exceeds the average food uptake per bee per day (37.7 mg/bee/day).

**TABLE 33 Mean consumption of feeding solution with and without consideration of evaporation in the 5 % acetone treated group**

| Mean Consumption of the Bees and Evaporation: 5 % Acetone | | | | | | |
|---|---|---|---|---|---|---|
| **Lab** | **Mean uptake per bee without evaporation** | **Mean evaporation per cage** | **Mean uptake per bee with evaporation** | **Mean evaporation per living bee** | **Difference** | |
| | **[mg/bee]** | **[mg/cage]** | **[mg/bee]** | **[mg/bee]** | **[%]** | **Type of feeder** |
| **1** | 28.9 | 61.4 | 19.0 | 9.9 | 34.2 | S |
| **2a** | 33.0 | 89.8 | 22.1 | 10.9 | 33.0 | S |
| **2b** | 32.9 | 106.9 | 25.3 | 7.6 | 23.2 | S |
| **3** | 44.0 | 71.9 | 36.7 | 7.4 | 16.7 | S |
| **4** | 39.1 | 135.4 | 20.5 | 18.6 | 47.5 | T |
| **5** | 44.0 | 373.3 | 4.7 | 39.3 | 89.3 | S |
| **6** | 28.2 | 35.1 | 23.5 | 4.7 | 16.7 | S |
| **7** | 36.9 | 99.6 | 26.9 | 10.0 | 27.1 | S |
| **8** | 34.7 | 105.6 | 21.5 | 13.2 | 38.1 | S |
| **9** | 31.9 | 81.4 | 23.5 | 8.4 | 26.4 | T |
| **10** | 37.3 | 89.2 | 27.8 | 9.5 | 25.5 | S |
| **11** | 37.0 | 87.2 | 25.6 | 11.3 | 30.6 | T |
| **12** | 48.1 | 92.2 | 38.1 | 10.0 | 20.8 | S |
| **13** | 29.7 | 123.6 | 17.8 | 11.9 | 40.0 | S |
| **14** | 36.3 | 75.2 | 26.8 | 9.5 | 26.2 | S |
| **15** | 33.1 | 35.5 | 25.8 | 7.3 | 22.0 | T |
| mean | **35.4** | **86.0** | **25.4** | **10.0** | **28.5** | |
| minimum | 28.2 | 35.1 | 17.8 | 4.7 | 16.7 | |
| maximum | 48.1 | 135.4 | 38.1 | 18.6 | 47.5 | |

*data from lab5a were not used due to incomprehensive data

S = Syringes, T = Tubes *e.g.* Eppendorf Tubes

110. Using 5 % acetone in the feeding solution led to a higher evaporation: a mean of 86.0 mg per day per cage weight loss was recorded from the feeders filled with 5 % acetone in sugar solution. This daily physical weight loss is accompanied by a daily uptake of 25.4 mg 5 % acetone/sugar solution. The minimum and maximum weight loss was 35.1 and 135.4 mg/day/cage, respectively (see TABLE 33).

111. Weight loss due to evaporation occurred in all tests and its level depends on factors like *e.g.* type of feeders, the kind of solvent and its volatility. Volatile solvents like acetone produce higher evaporation than solutions with lower volatility like water.

112. Apart from the official ring test, lab 1 made additional investigations regarding the relevance of evaporation in general and the correlation between the number of bees per cage and the observed evaporation. It was observed that the outcome of the evaporation on the food consumption depends on the number of bees in the respective cage. The relationship between food uptake and evaporation in correlation to the number of bees can be described in relative numbers as a ratio between mean evaporation per test cage per day and the food uptake. For each number of bees a specific ratio can be calculated. The following tables and figures show the relation between the number of bees and the evaporation for the untreated control (TABLE 34 and FIGURE 8) and the 5 % acetone control (TABLE 35 and FIGURE 9).

113. Taking the mean food uptake per day for the untreated control of 37.7 mg per bee per day (377 mg/day/10 bees; 339 mg/bee/day/9 days etc.) and the mean evaporation in the untreated control of 75.3 mg/day) the curve follows a flat and linear trend down to 5 or 4 bees and thereafter increases exponentially down to one bee (negative value: food uptake 37.7 mg/day/1 bee vs 75.3 mg/day evaporation = -37.6 weight loss of food).

114. The same trend can be observed for the evaporation pattern with 5 % acetone in sugar solution. As the evaporation is higher and the food consumption lower the trend is slightly stronger.

**TABLE 34 Ratio of evaporation per food uptake (in %) related to the number of bees for the untreated control (50 % w/v sucrose solution)**

| Number of bees/cage | Food uptake [mg/day] | Evaporation [mg/day] | Food uptake with evaporation [a] | Ratio evaporation/food uptake [%] |
|---|---|---|---|---|
| 10 | 376.8 | 75.3 | 301.5 | 20.0 |
| 9 | 339.1 | 75.3 | 263.8 | 22.2 |
| 8 | 301.4 | 75.3 | 226.1 | 25.0 |
| 7 | 263.8 | 75.3 | 188.5 | 28.5 |
| 6 | 226.1 | 75.3 | 150.8 | 33.3 |
| 5 | 188.4 | 75.3 | 113.1 | 40.0 |
| 4 | 150.7 | 75.3 | 75.4 | 50.0 |
| 3 | 113.0 | 75.3 | 37.7 | 66.6 |
| 2 | 75.4 | 75.3 | 0.1 | 99.9 |
| 1 | 37.7 | 75.3 | -37.6 | 199.8 |

[a] food uptake with Evaporation = Food uptake [mg/day] - Evaporation [mg/day]

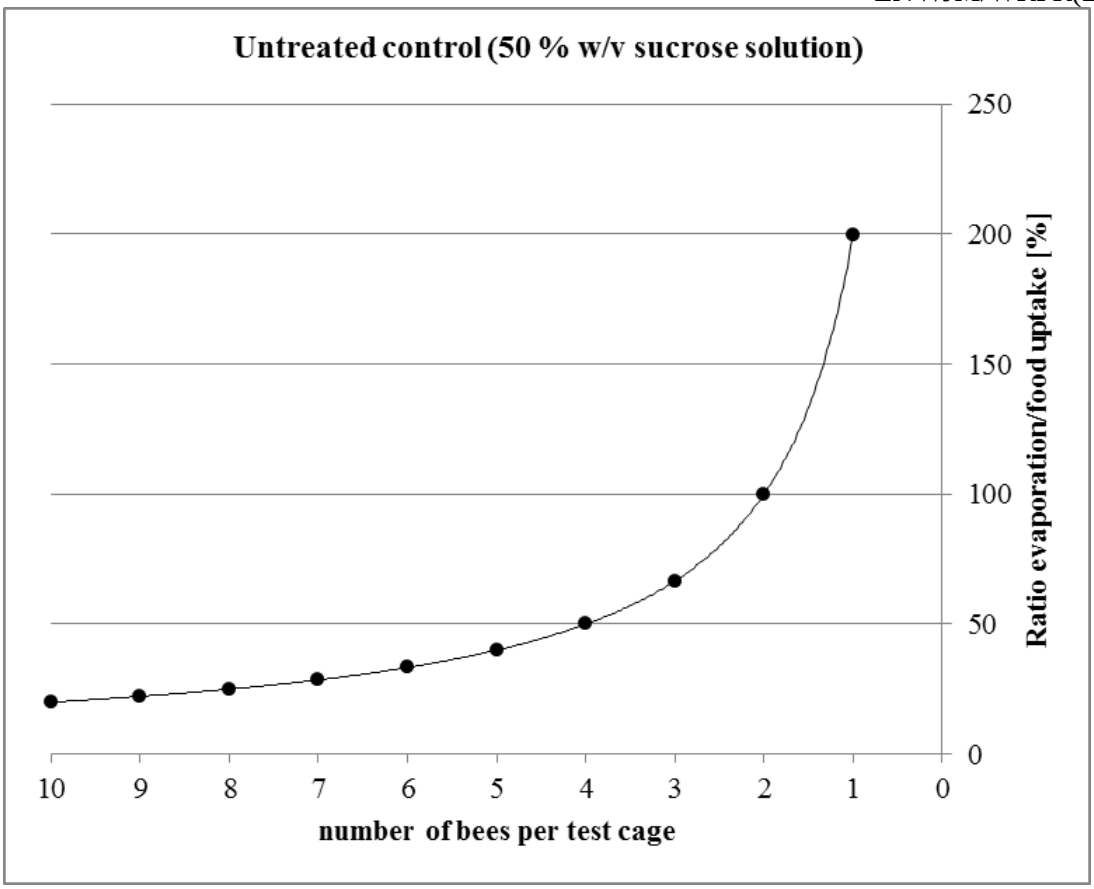


**FIGURE 8 Ratio of evaporation per food uptake (in %) related to the number of bees for the untreated control (50 % w/v sucrose solution)**

**TABLE 35 Ratio of evaporation per food uptake (in %) related to the number of bees for the 5 % acetone treatment group**

| Number of bees/cage | Food uptake [mg/day] | Evaporation [mg/day] | Food uptake with evaporation [a] | Ratio evaporation/food uptake [%] |
|---|---|---|---|---|
| 10 | 354.0 | 86.0 | 268.0 | 24.3 |
| 9 | 318.6 | 86.0 | 232.6 | 27.0 |
| 8 | 283.2 | 86.0 | 197.2 | 30.4 |
| 7 | 247.8 | 86.0 | 161.8 | 34.7 |
| 6 | 212.4 | 86.0 | 126.4 | 40.5 |
| 5 | 177.0 | 86.0 | 91.0 | 48.6 |
| 4 | 141.6 | 86.0 | 55.6 | 60.7 |
| 3 | 106.2 | 86.0 | 20.2 | 81.0 |
| 2 | 70.8 | 86.0 | -15.2 | 121.5 |
| 1 | 35.4 | 86.0 | -50.6 | 242.9 |

[a] Food uptake with Evaporation = Food uptake [mg/day] - Evaporation [mg/day]

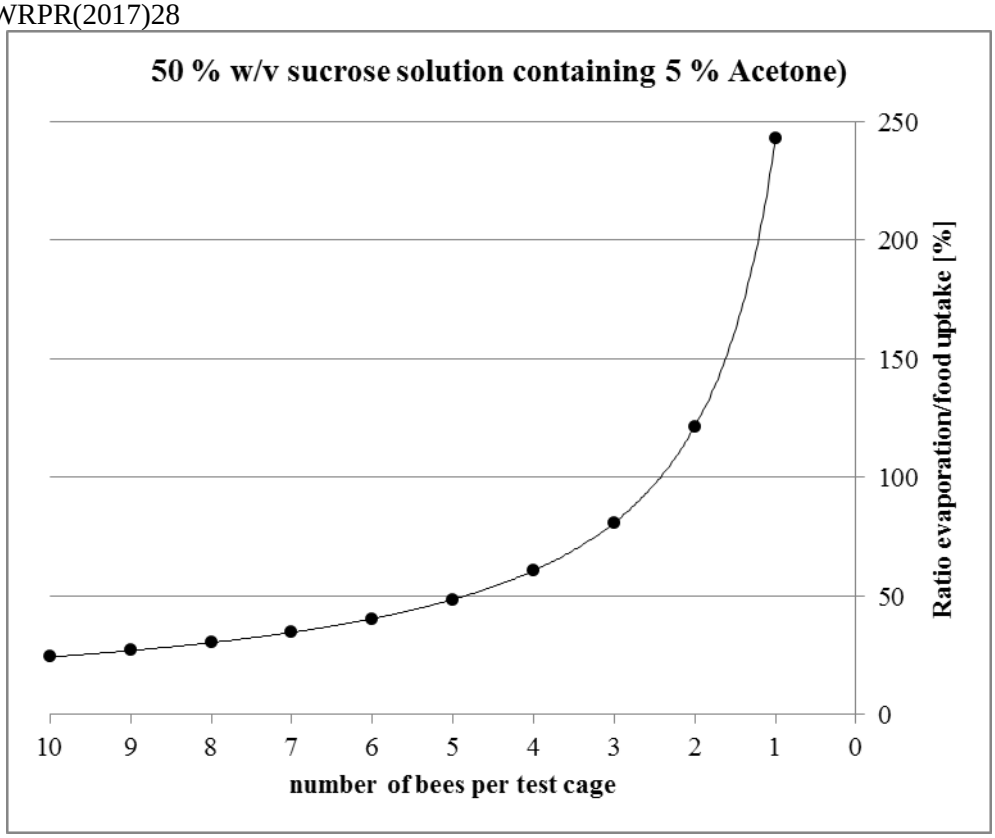


**FIGURE 9 Ratio of evaporation per food uptake (in %) related to the number of bees for the 5 % acetone treatment group**

**Possible Influence of the Number of Bees per Test Cage and possible Colony Effect on the Outcome of the Test**

115. Lab 16 conducted one experiment in order to assess the influence of the number of bees per test cage on the natural background mortality of the bees. This test also investigated a possible colony effect on control mortality.

116. Two chronic oral tests were run concurrently. One test was conducted with 10 bees per test cage and the other test was conducted with 50 bees per test cage. Test cages had the same size (12 x 6 x 8 cm) in both tests. Bees were provided with untreated sugar solution (50 % w/v) *ad libitum* for 10 days. Both tests were run with bees of 4 different colonies and with 3 test cages (replicates) per colony.

117. The results are presented in Table 9.

**TABLE 36 Mortality [%] in relation to the number of bees per cage and the use of different colonies**

| | | Days of Exposure | | | | | | | | | | Mortality | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hive number | number of bees/cage | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | Total number of dead bees | Cumulated mortality % | Mean | SD | Mean | SD |
| 76 | 10 | 1 | 0 | 0 | - | - | 1 | 0 | 0 | 0 | 0 | 2 | 20 | | | | |
| 76 | 10 | 0 | 0 | 0 | - | - | 0 | 1 | 0 | 0 | 0 | 1 | 10 | 20.0 | 10.0 | | |
| 76 | 10 | 1 | 0 | 0 | - | - | 0 | 0 | 0 | 1 | 1 | 3 | 30 | | | | |
| 78 | 10 | 0 | 0 | 0 | - | - | 0 | 0 | 0 | 0 | 2 | 2 | 20 | | | | |
| 78 | 10 | 0 | 0 | 0 | - | - | 0 | 0 | 0 | 0 | 1 | 1 | 10 | 13.3 | 5.8 | | |
| 78 | 10 | 0 | 0 | 1 | - | - | 0 | 0 | 0 | 0 | 0 | 1 | 10 | | | 14.2 | 10.0 |
| 79 | 10 | 0 | 0 | 0 | - | - | 0 | 0 | 1 | 0 | 0 | 1 | 10 | | | | |
| 79 | 10 | 0 | 0 | 0 | - | - | 0 | 0 | 1 | 0 | 0 | 1 | 10 | 13.3 | 5.8 | | |
| 79 | 10 | 0 | 0 | 0 | - | - | 0 | 0 | 0 | 1 | 1 | 2 | 20 | | | | |
| 84 | 10 | 0 | 0 | 0 | - | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | |
| 84 | 10 | 0 | 0 | 0 | - | - | 1 | 0 | 0 | 0 | 2 | 3 | 30 | 10.0 | 17.3 | | |
| 84 | 10 | 0 | 0 | 0 | - | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | |

| | | Days of Exposure | | | | | | | | | | Mortality | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hive number | number of bees/cage | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | Total number of dead bees | Cumulated mortality % | | SD | Mean | SD |
| 76 | 50 | 0 | 0 | 0 | - | - | 1 | 0 | 0 | 0 | 0 | 1 | 2 | | | | |
| 76 | 50 | 0 | 0 | 0 | - | - | 5 | 0 | 1 | 0 | 1 | 7 | 14 | 8.7 | 30.6 | | |
| 76 | 50 | 0 | 0 | 1 | - | - | 0 | 0 | 1 | 3 | 0 | 5 | 10 | | | | |
| 78 | 50 | 0 | 0 | 1 | - | - | 2 | 3 | 0 | 0 | 0 | 6 | 12 | | | | |
| 78 | 50 | 0 | 0 | 0 | - | - | 1 | 0 | 0 | 0 | 1 | 2 | 4 | 7.3 | 20.8 | | |
| 78 | 50 | 0 | 0 | 2 | - | - | 1 | 0 | 0 | 0 | 0 | 3 | 6 | | | 6.2 | 4.5 |
| 79 | 50 | 0 | 0 | 0 | - | - | 0 | 1 | 0 | 1 | 2 | 4 | 8 | | | | |
| 79 | 50 | 0 | 0 | 1 | - | - | 0 | 1 | 0 | 2 | 0 | 4 | 8 | 5.3 | 23.1 | | |
| 79 | 50 | 0 | 0 | 0 | - | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | |
| 84 | 50 | 0 | 0 | 0 | - | - | 1 | 1 | 0 | 1 | 0 | 3 | 6 | | | | |
| 84 | 50 | 0 | 0 | 0 | - | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.3 | 15.3 | | |
| 84 | 50 | 0 | 0 | 1 | - | - | 0 | 1 | 0 | 0 | 0 | 2 | 4 | | | | |

"-" = no assessment was conducted

*Number of bees:*

118. The test run with 10 bees per cage was conducted with 4 colonies each replicated 3 times with 10 bees, resulting in 12 single replicates and 120 bees on total. At the end of the test a mean control mortality of 14.2 % and a standard deviation of 10 % occurred.

119. The experiment with an increased number of bees per cage (50 bees per cage) was also conducted with bees from 4 colonies and 3 replicates per colony (on total 600 bees). This test run resulted in a final mortality of 6.2 % (SD 4.5 %).

120. Comparing both experiments, mortality in the test with the standard number of 10 bees per cage was higher compared to the test with an increased number of 50 per bees per cage.

121. A statistical analysis of the mortality data of both test groups shows a statistically significant difference between both experiments (Welch t-test, pairwise comparison, one-sided smaller, $\alpha = 0.05$ and Kruskal-Wallis-test Procedure, $\alpha = 0.05$).

122. Looking at the mean mortality of the untreated control groups of this 2nd international ring test, which was conducted with 10 bees per cage (mean: 3.1 %), the results of both additional experiments (10 and 50 bees/cage) showed an increased mortality in general. Even the mortality of the test conducted with 50 bees/cage, which was statistically significantly lower compared to the test group with 10 bees/cage, was higher than the mean mortality over all tests in the untreated control group with 10 bees/cage during the ring test conducted in 2015 (6.2 % compared to 3.1 %, respectively).

123. The very low control mortality observed in all trials in the 1st and 2nd ring test (altogether 35 tests in 2014 and 2015; mean mortality over the tests: 2.0 % and 3.1 %, respectively) indicated a stable and robust test system which produces consistent and reliable data with a number of 10 bees/cage.

124. The results of this single experiment must be seen as not representative but if appropriate further tests might be performed.

125. In this context it should also be considered that an increased number of bees per cage (> 10) will make it difficult or even impossible to conduct a proper quantitative assessment of any kind of observations *e.g.* behavioural impairments, counting of surviving bees. Increasing the number of bees from 10 to 50 bees per replicate will also increase the number of bees 5-fold which were used and killed by an experiment. This fact is disproportionate to the unclear benefit of using 50 bees per replicate.

*Colony Effect:*

Analysing the mortality data of the 2 tests, it can be concluded that no colony effect was observed.

126. Statistical analysis of the mortality data of both test groups (10 bees per cage and 50 bees per cage) didn't show a statistical significant difference when comparing the data from the 4 different colonies (One-way Analysis of Variance or Kruskal-Wallis-test Procedure, $\alpha = 0.05$).

## CONCLUSIONS

127. In this 2nd international OECD ring test, 16 laboratories from 9 countries have conducted chronic toxicity tests on honey bees in summer 2015. In total 19 data sets were provided. The labs followed a standardised protocol without major deviations. The aim of the ring test was, to assess possible side-effects of the solvent acetone and to investigate the occurrence and outcome of possible evaporation of the feeding solution. One additional test was conducted in order to assess the influence of the number of bees per test cage on the natural mortality of the bees during the 10 day test period. This test also investigated possible colony effects which might play a role when using bees of one versus several colonies for a test.

128. A validity criterion of ≤ 15 % for the untreated control mortality was set and met by all laboratories. This confirmed the results from the first ring test conducted in 2014 and demonstrated the feasibility and robustness of the test. Regarding the reference item treatment the observations of this ring test justify the testing of only one concentration of the reference item which results in a mortality of ≥ 50 % following chronic exposure at the end of the test period. Both validity criteria can be used for a standardized test guideline.

129. The results of these ring test showed, that the described test method is suitable to assess the chronic effects of plant protection products or other chemicals on honey bees in the laboratory.

130. In contrast to the results of the 1st ring test from 2014 it could be shown that acetone can be used as a solvent up to a concentration of 5 % in sugar solution.

131. A weight loss of feeding solution due to evaporation was observed in all tests conducted.

132. The quantity of food loss strongly differs among the laboratories. It is most likely that its extend is influenced by the following factors:

- type of feeder
- diameter of the access hole of the feeder
- relative humidity
- volatility of the solvent
- The outcome of the evaporation on the calculated food uptake of the bees depends on the number of bees per cage: evaporation has a lower impact if no mortality occurred (*i.e.* most bees survived in one cage), but the impact is considerable from 5 dead bees onwards.

133. Therefore it is recommended to reduce evaporation by technical arrangements and to adjust the calculation of the food consumption by the bees for evaporation. It is advised to report corrected food consumption values in order to determine realistic and conservative data on food consumption and likewise to avoid an overestimation of the actual food consumption by the bees.

## REFERENCES

CEB (Commission des Essais Biologiques), 2012. Méthode d'évaluation des effets de toxicité aiguë et á court terme des préparations phytopharmaceutiques sur l'abeille domestique (Apis mellifera L.). AFPP method n°230.

Kling, A. & Schmitzer, S. (2015): Proposal for a new OECD guideline for the testing of chemicals on adult honey bees (*Apis mellifera* L.) in a 10 day chronic feeding test in the laboratory and results of the recent ring test 2014. Hazards of pesticides to bees - 12th International Symposium of the ICP-PR Bee Protection Group, Ghent (Belgium), 15-17 September 2014. Julius-Kühn-Archiv, 450, pp. 69-74.

Niederdrenk, S., Oppinger, M., Kusebauch, B., BASF SE internal Report, 2014: Determination of Dimethoate in 50 % Sucrose Solution

OECD 213, 1998 Guideline for the testing of chemicals; Honey bees; acute oral toxicity test.

Report – Summary of the Results of a Test Conducted by several German Laboratories for the Standardisation of a Test Design (2013); Assessment of Chronic Effects to the Honeybee, *Apis mellifera* L., in a 10 Day Continuous Laboratory Feeding Test; compiled by Annette Kling, Stephan Schmitzer, Markus Barth, Dorothee Lüken, Nicole Hanewald, David Gladbach on behalf of the bee expert group „AG Bienenschutz", Germany

Schmitzer, S, Fieseler, A., IBACON internal Report, 2014: Determination of Dimethoate in 50 % Sucrose Solution

ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

# APPENDIX

**TABLE 37 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 1**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 |
| 1 % acetone | 0.0 | 0.0 | 3.3 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 |
| Reference item (1.0 mg a.i./kg) | 0.0 | 0.0 | 10.0 | 53.3 | 80.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 39.3 | 62.3 | 44.3 | 37.3 | 35.7 | 41.0 | 39.3 | 42.0 | 43.0 | 41.7 | **42.6** |
| 50 % sucrose solution containing 5 % acetone | 98.7 | 58.3 | 47.7 | 72.0 | 70.0 | 47.7 | 49.0 | 82.0 | 44.0 | 44.3 | **61.4** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 21.2 | 28.9 | 26.9 | 27.4 | 45.2 | 27.4 | 31.1 | 29.6 | 34.3 | 32.6 | **30.5** |
| 1 % acetone | 27.2 | 33.0 | 25.1 | 29.9 | 34.0 | 40.0 | 33.2 | 31.9 | 36.0 | 32.4 | **32.3** |
| 3 % acetone | 13.1 | 25.7 | 24.3 | 23.4 | 27.1 | 37.4 | 23.1 | 30.2 | 26.0 | 25.2 | **25.5** |
| 5 % acetone | 20.8 | 27.6 | 26.0 | 26.1 | 31.1 | 35.2 | 29.5 | 33.4 | 27.2 | 32.5 | **28.9** |
| Reference item (1.0 mg a.i./kg) | 19.0 | 23.4 | 12.8 | 24.1 | 23.4 | 36.9 | - | - | - | - | **23.3** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 17.3 | 22.7 | 22.5 | 23.7 | 41.6 | 23.3 | 27.2 | 25.4 | 30.0 | 28.2 | **26.2** |
| 1 % acetone | 23.3 | 26.8 | 20.7 | 26.0 | 30.1 | 35.5 | 28.9 | 27.3 | 31.3 | 27.9 | **27.8** |
| 3 % acetone | 9.2 | 19.5 | 19.9 | 19.7 | 23.5 | 33.3 | 19.2 | 26.0 | 21.7 | 21.0 | **21.3** |
| 5 % acetone | 16.9 | 21.4 | 21.6 | 22.4 | 27.5 | 31.1 | 25.6 | 29.2 | 22.9 | 28.3 | **24.7** |
| Reference item (1.0 mg a.i./kg) | 15.1 | 17.2 | 8.4 | 19.9 | 15.1 | 6.2 | - | - | - | - | **13.6** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 10.9 | 17.7 | 16.1 | 16.2 | 21.2 | 25.3 | 19.6 | 23.5 | 17.3 | 22.6 | **19.0** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (1.0 mg a.i./kg) | 0.019 | 0.023 | 0.013 | 0.024 | 0.023 | 0.037 | - | - | - | - | **0.023** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (1.0 mg a.i./kg) | 0.017 | 0.017 | 0.008 | 0.02 | 0.015 | 0.006 | - | - | - | - | **0.010** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (1.0 mg a.i./kg) | 0.019 | 0.042 | 0.055 | 0.079 | 0.102 | 0.139 | 0.139 | 0.139 | 0.139 | 0.139 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (1.0 mg a.i./kg) | 0.015 | 0.032 | 0.04 | 0.06 | 0.075 | 0.081 | 0.081 | 0.081 | 0.081 | 0.081 |

**TABLE 38 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 2a**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 13.3 | 13.3 | 13.3 | 16.7 | 20.0 |
| 5 % acetone | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 |
| Reference item (0.9 mg a.i./kg) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10.0 | 80.0 | 96.7 | 100.0 | 100.0 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 78.7 | 63.0 | 62.7 | 67.3 | 64.0 | 73.3 | 85.3 | 66.7 | 70.0 | 69.7 | **70.1** |
| 50 % sucrose solution containing 5 % acetone | 105.7 | 82.7 | 88.0 | 86.7 | 71.7 | 94.0 | 80.7 | 87.0 | 96.7 | 105.0 | **89.8** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 29.4 | 25.0 | 28.5 | 38.9 | 25.6 | 33.6 | 35.9 | 39.6 | 41.7 | 28.8 | **32.7** |
| 1 % acetone | 27.5 | 32.0 | 38.3 | 25.9 | 29.6 | 34.5 | 27.8 | 31.3 | 40.6 | 36.8 | **32.4** |
| 3 % acetone | 26.9 | 22.8 | 29.1 | 24.5 | 26.3 | 38.4 | 32.7 | 43.1 | 49.1 | 37.6 | **33.1** |
| 5 % acetone | 27.7 | 33.4 | 28.9 | 25.9 | 28.6 | 40.2 | 25.2 | 33.8 | 41.4 | 45.1 | **33.0** |
| Reference item (0.9 mg a.i./kg) | 25.5 | 29.9 | 15.7 | 25.7 | 10.1 | 18.3 | 19.9 | 18.2 | 72.0 | - | **24.0** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 21.5 | 18.7 | 22.2 | 32.2 | 19.2 | 26.3 | 27.4 | 32.9 | 34.7 | 21.8 | **25.7** |
| 1 % acetone | 19.6 | 25.7 | 32.0 | 19.2 | 23.2 | 27.2 | 19.3 | 24.6 | 33.6 | 29.8 | **25.4** |
| 3 % acetone | 19.0 | 16.5 | 22.8 | 17.8 | 19.9 | 31.1 | 22.3 | 34.9 | 40.6 | 28.3 | **25.3** |
| 5 % acetone | 17.1 | 22.8 | 17.9 | 15.0 | 17.6 | 29.2 | 14.2 | 22.9 | 30.4 | 34.1 | **22.1** |
| Reference item (0.9 mg a.i./kg) | 17.6 | 23.6 | 9.4 | 19.0 | 3.7 | 11.0 | 10.4 | 7.1 | 2.0 | - | **13.1** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 17.1 | 22.8 | 17.9 | 15.0 | 17.6 | 29.2 | 14.2 | 22.9 | 30.4 | 34.1 | **22.1** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (0.9 mg a.i./kg) | 0.023 | 0.027 | 0.015 | 0.024 | 0.009 | 0.017 | 0.018 | 0.016 | 0.065 | - | **0.022** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (0.9 mg a.i./kg) | 0.021 | 0.021 | 0.009 | 0.018 | 0.004 | 0.01 | 0.01 | 0.006 | 0.002 | - | **0.010** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.9 mg a.i./kg) | 0.023 | 0.05 | 0.065 | 0.089 | 0.098 | 0.115 | 0.133 | 0.149 | 0.214 | 0.214 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.9 mg a.i./kg) | 0.016 | 0.037 | 0.046 | 0.064 | 0.068 | 0.078 | 0.088 | 0.094 | 0.096 | 0.096 |

**TABLE 39 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 2b**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 |
| Reference item (0.9 mg a.i./kg) | 0.0 | 0.0 | 0.0 | 16.7 | 20.0 | 20.0 | 46.7 | 83.3 | 96.7 | 100.0 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 68.3 | 70.3 | 70.3 | 78.7 | 73.0 | 92.3 | 85.3 | 78.7 | 75.0 | 71.3 | **76.3** |
| 50 % sucrose solution containing 5 % acetone | 105.0 | 105.7 | 116.0 | 94.7 | 116.0 | 110.7 | 112.7 | 108.7 | 101.0 | 98.0 | **106.8** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 30.0 | 42.2 | 46.7 | 54.4 | 45.0 | 56.8 | 44.1 | 46.8 | 35.7 | 40.2 | **44.2** |
| 1 % acetone | 29.0 | 39.4 | 33.0 | 38.4 | 40.0 | 39.7 | 30.9 | 30.0 | 36.2 | 40.8 | **35.8** |
| 3 % acetone | 28.5 | 41.7 | 39.8 | 33.7 | 45.5 | 41.5 | 43.6 | 33.5 | 35.8 | 42.7 | **38.6** |
| 5 % acetone | 23.1 | 31.7 | 37.7 | 38.0 | 36.1 | 28.5 | 32.0 | 33.0 | 30.0 | 38.6 | **32.9** |
| Reference item (0.9 mg a.i./kg) | 28.2 | 33.4 | 38.8 | 22.2 | 16.0 | 21.6 | 29.5 | 35.5 | 53.2 | 77.0 | **31.8** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 23.2 | 35.2 | 39.7 | 46.5 | 37.7 | 47.3 | 35.3 | 38.7 | 27.9 | 32.8 | **36.4** |
| 1 % acetone | 22.2 | 32.4 | 26.0 | 30.5 | 32.7 | 30.5 | 22.4 | 22.1 | 28.7 | 33.7 | **28.1** |
| 3 % acetone | 21.7 | 34.7 | 32.8 | 25.8 | 38.2 | 32.3 | 35.1 | 25.6 | 28.3 | 35.6 | **31.0** |
| 5 % acetone | 16.3 | 24.7 | 30.7 | 30.1 | 28.8 | 19.3 | 23.5 | 25.1 | 22.5 | 31.5 | **25.2** |
| Reference item (0.9 mg a.i./kg) | 21.4 | 26.4 | 31.8 | 14.3 | 7.0 | 9.8 | 18.6 | 14.9 | 21.9 | 5.7 | **17.9** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 12.6 | 21.2 | 27.2 | 27.5 | 25.6 | 18.0 | 21.5 | 22.5 | 19.5 | 28.1 | **22.4** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (0.9 mg a.i./kg) | 0.025 | 0.03 | 0.035 | 0.02 | 0.014 | 0.02 | 0.027 | 0.032 | 0.048 | 0.069 | **0.029** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (0.9 mg a.i./kg) | 0.024 | 0.024 | 0.028 | 0.013 | 0.006 | 0.009 | 0.017 | 0.014 | 0.02 | 0.005 | **0.020** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.9 mg a.i./kg) | 0.025 | 0.055 | 0.09 | 0.11 | 0.124 | 0.144 | 0.171 | 0.203 | 0.251 | 0.32 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.9 mg a.i./kg) | 0.019 | 0.043 | 0.071 | 0.084 | 0.09 | 0.099 | 0.116 | 0.13 | 0.15 | 0.155 |

**TABLE 40 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 3**

| | Mortality in % | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | |
| Reference item (0.9 mg a.i./kg) | 0.0 | 0.0 | 10.0 | 23.3 | 80.0 | 93.3 | 96.7 | 100.0 | 100.0 | 100.0 | |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 44.0 | 46.7 | 57.4 | 51.3 | 134.0 | 49.2 | 66.7 | 88.3 | 78.2 | 30.9 | **64.7** |
| 50 % sucrose solution containing 5 % acetone | 73.5 | 58.2 | 69.3 | 73.0 | 154.9 | 60.3 | 53.2 | 70.2 | 68.6 | 38.0 | **71.9** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 25.1 | 41.9 | 41.9 | 48.4 | 48.5 | 47.8 | 57.9 | 73.8 | 72.8 | 81.7 | **54.0** |
| 1 % acetone | 24.4 | 41.3 | 38.5 | 36.1 | 47.0 | 50.0 | 65.8 | 64.3 | 74.6 | 60.8 | **50.3** |
| 3 % acetone | 26.4 | 36.7 | 48.4 | 51.9 | 43.0 | 64.5 | 70.8 | 67.1 | 75.3 | 86.0 | **57.0** |
| 5 % acetone | 30.5 | 39.9 | 45.9 | 34.3 | 41.3 | 44.9 | 50.0 | 56.7 | 52.9 | 43.7 | **44.0** |
| Reference item (0.9 mg a.i./kg) | 27.4 | 33.7 | 22.3 | 34.1 | 25.7 | 27.9 | 22.1 | 72.6 | - | - | **30.7** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 20.7 | 37.2 | 36.2 | 43.3 | 35.1 | 42.9 | 51.2 | 65.0 | 65.0 | 78.6 | **47.5** |
| 1 % acetone | 20.0 | 36.7 | 32.8 | 31.0 | 33.6 | 45.1 | 59.1 | 55.5 | 66.7 | 57.7 | **43.8** |
| 3 % acetone | 22.0 | 32.0 | 42.7 | 46.8 | 29.6 | 59.5 | 63.9 | 57.9 | 67.2 | 82.8 | **50.4** |
| 5 % acetone | 23.1 | 32.5 | 38.6 | 26.9 | 34.0 | 37.6 | 42.6 | 49.3 | 45.5 | 36.4 | **36.7** |
| Reference item (0.9 mg a.i./kg) | 23.0 | 29.1 | 16.5 | 28.3 | 7.4 | 19.7 | -11.3 | -15.7 | - | - | **17.0** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 23.1 | 32.5 | 38.6 | 26.9 | 34.0 | 37.6 | 42.6 | 49.3 | 45.5 | 36.4 | **36.7** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | DD |
| Reference item (0.9 mg a.i./kg) | 0.024 | 0.03 | 0.02 | 0.031 | 0.023 | 0.025 | 0.02 | 0.065 | - | - | **0.028** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | DD |
| Reference item (0.9 mg a.i./kg) | 0.026 | 0.026 | 0.015 | 0.026 | 0.007 | 0.018 | -0.01 | -0.014 | - | - | **0.020** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.9 mg a.i./kg) | 0.024 | 0.054 | 0.074 | 0.105 | 0.128 | 0.153 | 0.173 | 0.238 | 0.238 | 0.238 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.9 mg a.i./kg) | 0.02 | 0.046 | 0.061 | 0.087 | 0.094 | 0.112 | 0.102 | 0.088 | 0.088 | 0.088 |

**TABLE 41 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 4**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 6.7 | 6.7 | 10.0 | 10.0 |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Reference item (0.6 mg a.i./kg) | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 6.7 | 43.3 | 70.0 | 90.0 | 100.0 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 132.3 | 115.3 | 109.3 | 120.0 | 108.3 | 119.3 | 94.3 | 118.3 | 103.3 | 96.7 | **111.7** |
| 50 % sucrose solution containing 5 % acetone | 185.7 | 156.0 | 134.3 | 127.7 | 132.0 | 158.7 | 115.3 | 134.3 | 101.7 | 108.0 | **135.4** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 38.4 | 37.5 | 37.9 | 33.6 | 31.7 | 53.4 | 32.8 | 36.5 | 35.4 | 52.6 | **39.0** |
| 1 % acetone | 34.7 | 46.2 | 31.1 | 29.9 | 30.3 | 31.6 | 31.6 | 43.4 | 28.9 | 35.7 | **34.4** |
| 3 % acetone | 48.6 | 42.3 | 23.5 | 35.0 | 33.0 | 44.5 | 32.4 | 38.0 | 40.2 | 39.1 | **37.7** |
| 5 % acetone | 44.5 | 43.7 | 31.7 | 32.8 | 32.8 | 51.4 | 35.4 | 42.5 | 35.8 | 40.6 | **39.1** |
| Reference item (0.6 mg a.i./kg) | 38.3 | 38.3 | 25.2 | 28.6 | 31.2 | 46.6 | 18.2 | 52.2 | 70.8 | 71.3 | **41.1** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 25.2 | 26.0 | 27.0 | 21.6 | 20.9 | 41.5 | 23.4 | 24.2 | 24.7 | 42.5 | **27.7** |
| 1 % acetone | 21.5 | 34.7 | 20.2 | 17.9 | 19.5 | 19.3 | 21.9 | 30.6 | 17.7 | 24.8 | **22.8** |
| 3 % acetone | 35.4 | 30.8 | 12.6 | 23.0 | 22.2 | 32.6 | 23.0 | 26.2 | 29.9 | 29.4 | **26.5** |
| 5 % acetone | 25.9 | 25.1 | 13.1 | 14.2 | 14.2 | 32.8 | 16.8 | 23.9 | 17.2 | 22.0 | **20.5** |
| Reference item (0.6 mg a.i./kg) | 25.1 | 26.8 | 14.3 | 16.6 | 20.4 | 34.2 | 8.1 | 27.6 | 19.2 | -1.3 | **19.8** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 25.9 | 25.1 | 13.1 | 14.2 | 14.2 | 32.8 | 16.8 | 23.9 | 17.2 | 22.0 | **20.5** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | DD |
| Reference item (0.6 mg a.i./kg) | 0.023 | 0.023 | 0.015 | 0.017 | 0.019 | 0.028 | 0.011 | 0.031 | 0.043 | 0.043 | **0.025** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | DD |
| Reference item (0.6 mg a.i./kg) | 0.016 | 0.016 | 0.009 | 0.01 | 0.012 | 0.02 | 0.005 | 0.017 | 0.011 | -0.001 | **0.010** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.6 mg a.i./kg) | 0.023 | 0.046 | 0.061 | 0.078 | 0.097 | 0.125 | 0.136 | 0.167 | 0.21 | 0.253 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.6 mg a.i./kg) | 0.015 | 0.031 | 0.04 | 0.05 | 0.062 | 0.082 | 0.087 | 0.104 | 0.115 | 0.114 |

**TABLE 42 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 5**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 3.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 6.7 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 13.3 |
| Reference item (1 mg a.i./kg) | 100.0 | | | | | | | | | |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 97.7 | 111.7 | 114.0 | 107.3 | 116.0 | 101.3 | 94.3 | 108.0 | 114.7 | 169.7 | **113.5** |
| 50 % sucrose solution containing 5 % acetone | 401.7 | 396.0 | 366.0 | 402.7 | 336.7 | 336.0 | 336.3 | 321.3 | 402.7 | 433.3 | **373.3** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 25.7 | 39.5 | 33.5 | 40.1 | 35.7 | 42.6 | 33.2 | 44.4 | 35.4 | 46.5 | **37.7** |
| 1 % acetone | 24.3 | 42.0 | 39.7 | 37.4 | 43.6 | 40.0 | 36.7 | 38.7 | 44.5 | 54.8 | **40.2** |
| 3 % acetone | 38.5 | 35.1 | 41.0 | 42.3 | 37.7 | 41.7 | 36.0 | 47.2 | 49.8 | 59.2 | **42.8** |
| 5 % acetone | 38.6 | 47.7 | 39.3 | 42.1 | 35.7 | 42.4 | 38.1 | 48.1 | 51.2 | 56.8 | **44.0** |
| Reference item (1 mg a.i./kg) | 9.7 | - | - | - | - | - | - | - | - | - | **9.7** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 15.9 | 28.3 | 21.7 | 29.4 | 24.1 | 32.5 | 23.8 | 33.6 | 23.9 | 29.5 | **26.3** |
| 1 % acetone | 14.5 | 30.8 | 28.3 | 26.7 | 32.0 | 29.9 | 27.3 | 27.9 | 33.0 | 37.8 | **28.8** |
| 3 % acetone | 28.7 | 23.9 | 29.6 | 31.6 | 26.1 | 31.6 | 26.6 | 36.4 | 37.9 | 41.6 | **31.4** |
| 5 % acetone | -1.6 | 7.5 | -0.9 | 1.9 | -4.5 | 2.2 | -2.1 | 7.9 | 11.0 | 16.6 | **3.8** |
| Reference item (1 mg a.i./kg) | -0.1 | - | - | - | - | - | - | - | - | - | **-0.1** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | -1.6 | 7.5 | -0.9 | 1.9 | -4.5 | 2.2 | -2.1 | 7.9 | 11.0 | 16.6 | **3.8** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | DD |
| Reference item (1 mg a.i./kg) | 0.01 | - | - | - | - | - | - | - | - | - | **0.010** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | DD |
| Reference item (1 mg a.i./kg) | - | - | - | - | - | - | - | - | - | - | **0.000** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (1 mg a.i./kg) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (1 mg a.i./kg) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**TABLE 43 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 6**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 6.7 |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 |
| Reference item (1.0 mg a.i./kg) | 0.0 | 0.0 | 13.3 | 53.3 | 63.3 | 96.7 | 96.7 | 100.0 | 100.0 | 100.0 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 55.5 | 38.8 | 43.3 | 39.4 | 52.3 | 42.5 | 44.9 | 46.5 | 36.8 | 34.5 | **43.5** |
| 50 % sucrose solution containing 5 % acetone | 44.6 | 29.7 | 34.4 | 35.7 | 34.1 | 32.1 | 39.2 | 41.4 | 28.2 | 31.4 | **35.1** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 25.9 | 22.6 | 35.7 | 34.5 | 28.9 | 41.3 | 37.7 | 43.1 | 23.3 | 24.4 | **31.7** |
| 1 % acetone | 24.7 | 21.5 | 22.6 | 35.3 | 21.3 | 30.2 | 26.2 | 40.1 | 17.6 | 29.3 | **26.9** |
| 3 % acetone | 21.7 | 28.2 | 27.0 | 39.1 | 27.3 | 31.4 | 25.0 | 33.5 | 21.2 | 26.2 | **28.1** |
| 5 % acetone | 28.7 | 27.4 | 31.0 | 25.7 | 38.2 | 29.2 | 25.3 | 40.1 | 10.9 | 25.8 | **28.2** |
| Reference item (1.0 mg a.i./kg) | 26.4 | 26.1 | 22.2 | 27.9 | 27.2 | 36.0 | 83.0 | 39.5 | - | - | **30.7** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 20.3 | 18.7 | 31.4 | 30.6 | 23.6 | 36.4 | 32.6 | 37.8 | 19.1 | 20.5 | **27.1** |
| 1 % acetone | 19.1 | 17.6 | 18.3 | 31.3 | 16.1 | 25.8 | 21.5 | 35.2 | 13.8 | 25.7 | **22.4** |
| 3 % acetone | 16.1 | 24.3 | 22.6 | 35.1 | 22.0 | 27.2 | 20.5 | 28.7 | 17.3 | 22.7 | **23.7** |
| 5 % acetone | 24.2 | 22.9 | 26.6 | 21.3 | 33.7 | 24.8 | 20.9 | 34.1 | 6.1 | 20.5 | **23.5** |
| Reference item (1.0 mg a.i./kg) | 20.9 | 22.2 | 17.9 | 23.2 | 2.2 | 12.6 | 38.1 | -7.0 | - | - | **16.6** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 24.2 | 22.9 | 26.6 | 21.3 | 33.7 | 24.8 | 20.9 | 34.1 | 6.1 | 20.5 | **23.5** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (1.0 mg a.i./kg) | 0.027 | 0.026 | 0.022 | 0.028 | 0.027 | 0.036 | 0.083 | 0.04 | - | - | **0.031** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (1.0 mg a.i./kg) | 0.022 | 0.022 | 0.018 | 0.023 | 0.002 | 0.013 | 0.038 | -0.007 | - | - | **0.020** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (1.0 mg a.i./kg) | 0.027 | 0.053 | 0.075 | 0.103 | 0.13 | 0.166 | 0.249 | 0.289 | 0.289 | 0.289 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (1.0 mg a.i./kg) | 0.021 | 0.043 | 0.061 | 0.084 | 0.086 | 0.099 | 0.137 | 0.13 | 0.13 | 0.13 |

**TABLE 44 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 7a**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 99.7 | 83.3 | 111.0 | 102.0 | 101.3 | 90.7 | 103.7 | 101.7 | 107.7 | 87.3 | **98.7** |
| 50 % sucrose solution containing 5 % acetone | 100.3 | 93.3 | 111.0 | 66.0 | 113.3 | 92.0 | 111.3 | 113.7 | 102.7 | 92.3 | **99.6** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 27.3 | 39.9 | 59.9 | 25.5 | 44.9 | 56.5 | 37.6 | 54.8 | 35.9 | 33.8 | **41.6** |
| 5 % acetone | 24.4 | 33.3 | 49.8 | 29.3 | 43.9 | 43.0 | 31.9 | 41.0 | 34.5 | 37.5 | **36.9** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 17.3 | 31.6 | 48.8 | 15.3 | 34.8 | 47.1 | 26.8 | 44.3 | 24.7 | 24.8 | **31.5** |
| 5 % acetone | 14.4 | 23.3 | 39.8 | 19.3 | 33.9 | 33.0 | 21.9 | 31.0 | 24.5 | 27.5 | **26.9** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 14.4 | 23.3 | 39.8 | 19.3 | 33.9 | 33.0 | 21.9 | 31.0 | 24.5 | 27.5 | **26.9** |

**TABLE 45 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 7b**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution containing 5 % acetone | 106.3 | 107.3 | 106.0 | 124.0 | 91.3 | 98.7 | 111.0 | 85.3 | 97.0 | 108.3 | **103.5** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 19.4 | 27.8 | 52.5 | 39.9 | 30.1 | 20.6 | 48.4 | 48.2 | 21.4 | 49.7 | **37.2** |
| 5 % acetone | 27.9 | 24.7 | 45.8 | 43.6 | 27.5 | 34.4 | 39.5 | 19.7 | 25.9 | 38.6 | **32.5** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 10.6 | 25.7 | 16.7 | 32.0 | 16.6 | 27.7 | 25.3 | 19.8 | 23.1 | 21.8 | **21.9** |

**TABLE 46 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 8**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Reference item (0.9 mg a.i./kg) | 0.0 | 3.3 | 16.7 | 40.0 | 76.7 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 101.5 | 100.1 | 92.0 | 91.7 | 138.0 | 90.5 | 81.4 | 91.6 | 109.4 | 89.0 | **98.5** |
| 50 % sucrose solution containing 5 % acetone | 132.2 | 113.3 | 104.3 | 91.6 | 153.7 | 86.4 | 92.3 | 89.3 | 97.9 | 94.5 | **105.6** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 34.8 | 33.0 | 36.3 | 19.8 | 42.2 | 34.8 | 34.5 | 34.6 | 28.8 | 33.6 | **33.2** |
| 1 % acetone | 29.5 | 35.3 | 32.8 | 32.4 | 43.0 | 30.7 | 41.2 | 40.3 | 29.7 | 42.0 | **35.7** |
| 3 % acetone | 29.9 | 29.8 | 38.4 | 28.2 | 39.5 | 30.5 | 39.4 | 30.4 | 23.7 | 36.9 | **32.7** |
| 5 % acetone | 24.7 | 37.4 | 27.7 | 30.3 | 52.5 | 30.0 | 39.9 | 36.6 | 31.5 | 36.8 | **34.7** |
| Reference item (0.9 mg a.i./kg) | 31.7 | 26.1 | 24.1 | 35.6 | 24.5 | 63.2 | - | - | - | - | **34.2** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 24.6 | 23.0 | 27.1 | 10.6 | 28.4 | 25.4 | 26.1 | 25.1 | 17.5 | 24.3 | **23.2** |
| 1 % acetone | 19.4 | 25.3 | 23.6 | 23.2 | 29.2 | 21.7 | 33.1 | 31.1 | 18.8 | 33.1 | **25.8** |
| 3 % acetone | 19.8 | 19.8 | 29.2 | 19.0 | 25.7 | 21.5 | 31.3 | 21.2 | 12.7 | 28.0 | **22.8** |
| 5 % acetone | 11.5 | 24.1 | 14.5 | 17.0 | 39.3 | 16.7 | 26.7 | 23.4 | 18.3 | 23.6 | **21.5** |
| Reference item (0.9 mg a.i./kg) | 21.5 | 16.1 | 14.5 | 24.3 | 1.1 | 10.4 | - | - | - | - | **14.7** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 11.5 | 24.1 | 14.5 | 17.0 | 39.3 | 16.7 | 26.7 | 23.4 | 18.3 | 23.6 | **21.5** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (0.9 mg a.i./kg) | 0.029 | 0.023 | 0.022 | 0.032 | 0.022 | 0.057 | - | - | - | - | **0.031** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (0.9 mg a.i./kg) | 0.014 | 0.014 | 0.013 | 0.022 | 0.001 | 0.009 | - | - | - | - | **0.010** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.9 mg a.i./kg) | 0.029 | 0.052 | 0.074 | 0.106 | 0.128 | 0.185 | 0.185 | 0.185 | 0.185 | 0.185 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.9 mg a.i./kg) | 0.02 | 0.034 | 0.047 | 0.069 | 0.07 | 0.079 | 0.079 | 0.079 | 0.079 | 0.079 |

**TABLE 47 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 9**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 10.0 | 10.0 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 6.7 | 6.7 |
| Reference item (0.8 mg a.i./kg) | 0.0 | 3.3 | 3.3 | 6.7 | 13.3 | 50.0 | 100.0 | 100.0 | 100.0 | 100.0 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 75.3 | 72.3 | 70.3 | 60.0 | 60.0 | 63.7 | 60.0 | 68.0 | 59.7 | 61.7 | **65.1** |
| 50 % sucrose solution containing 5 % acetone | 82.3 | 89.7 | 96.7 | 84.7 | 79.0 | 73.7 | 82.0 | 83.0 | 78.0 | 65.0 | **81.4** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 26.7 | 36.1 | 26.1 | 34.7 | 34.0 | 32.6 | 45.7 | 37.4 | 39.5 | 34.1 | **34.7** |
| 1 % acetone | 27.0 | 35.2 | 28.8 | 37.0 | 31.6 | 36.4 | 39.0 | 32.7 | 36.8 | 38.0 | **34.3** |
| 3 % acetone | 32.4 | 31.0 | 34.2 | 32.2 | 33.3 | 38.0 | 41.9 | 35.3 | 37.5 | 32.9 | **34.9** |
| 5 % acetone | 22.5 | 30.6 | 29.3 | 29.2 | 27.8 | 37.8 | 40.4 | 27.9 | 41.0 | 32.7 | **31.9** |
| Reference item (0.8 mg a.i./kg) | 31.5 | 30.3 | 26.8 | 30.9 | 20.2 | 27.6 | 20.7 | - | - | - | **26.9** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 19.2 | 28.9 | 19.1 | 28.7 | 28.0 | 26.2 | 39.7 | 30.6 | 33.5 | 27.9 | **28.2** |
| 1 % acetone | 19.5 | 28.0 | 21.8 | 31.0 | 25.6 | 30.0 | 33.0 | 25.9 | 30.8 | 31.8 | **27.7** |
| 3 % acetone | 24.9 | 23.8 | 27.2 | 26.2 | 27.3 | 31.6 | 35.6 | 28.2 | 31.3 | 25.9 | **28.2** |
| 5 % acetone | 14.3 | 22.4 | 21.1 | 21.0 | 19.3 | 29.3 | 31.9 | 19.4 | 32.5 | 23.8 | **23.5** |
| Reference item (0.8 mg a.i./kg) | 24.0 | 23.1 | 19.6 | 24.6 | 13.7 | 20.2 | 7.9 | - | - | - | **19.0** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 14.3 | 22.4 | 21.1 | 21.0 | 19.3 | 29.3 | 31.9 | 19.4 | 32.5 | 23.8 | **23.5** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (0.8 mg a.i./kg) | 0.025 | 0.025 | 0.022 | 0.025 | 0.016 | 0.022 | 0.017 | - | - | - | **0.022** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (0.8 mg a.i./kg) | 0.019 | 0.019 | 0.016 | 0.02 | 0.011 | 0.016 | 0.006 | - | - | - | **0.020** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.8 mg a.i./kg) | 0.025 | 0.05 | 0.072 | 0.097 | 0.113 | 0.135 | 0.152 | 0.152 | 0.152 | 0.152 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.8 mg a.i./kg) | 0.019 | 0.038 | 0.054 | 0.074 | 0.085 | 0.101 | 0.107 | 0.107 | 0.107 | 0.107 |

**TABLE 48 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 10**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Reference item (0.702 mg a.i./kg) | 0.0 | 0.0 | 0.0 | 3.3 | 6.7 | 13.3 | 33.3 | 56.7 | 90.0 | 100.0 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 61.7 | 63.7 | 62.0 | 56.3 | 63.3 | 64.7 | 78.7 | 63.0 | 68.3 | 64.0 | **64.6** |
| 50 % sucrose solution containing 5 % acetone | 95.0 | 92.3 | 92.0 | 99.3 | 83.7 | 89.3 | 90.0 | 95.7 | 74.7 | 79.7 | **89.2** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 21.1 | 28.3 | 36.4 | 33.8 | 37.4 | 34.6 | 39.2 | 25.6 | 34.3 | 33.2 | **32.4** |
| 1 % acetone | 21.3 | 20.5 | 36.9 | 35.2 | 40.4 | 34.0 | 37.5 | 42.5 | 28.0 | 40.9 | **33.7** |
| 3 % acetone | 21.2 | 32.4 | 33.4 | 33.6 | 34.7 | 34.6 | 29.5 | 32.3 | 33.6 | 34.0 | **31.9** |
| 5 % acetone | 21.9 | 33.7 | 36.4 | 41.8 | 40.6 | 37.3 | 45.3 | 38.8 | 36.7 | 40.0 | **37.3** |
| Reference item (0.702 mg a.i./kg) | 23.6 | 32.2 | 29.6 | 19.0 | 23.1 | 20.5 | 20.3 | 23.9 | 30.5 | 28.0 | **24.9** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 14.9 | 21.9 | 30.2 | 28.2 | 31.1 | 28.1 | 31.3 | 19.3 | 27.5 | 26.8 | **25.9** |
| 1 % acetone | 15.1 | 14.1 | 30.7 | 29.6 | 34.1 | 27.5 | 29.6 | 36.2 | 21.2 | 34.5 | **27.3** |
| 3 % acetone | 15.0 | 26.0 | 27.2 | 28.0 | 28.4 | 28.1 | 21.6 | 26.0 | 26.8 | 27.6 | **25.5** |
| 5 % acetone | 12.4 | 24.2 | 26.9 | 32.3 | 31.1 | 27.8 | 35.8 | 29.3 | 27.2 | 30.5 | **27.8** |
| Reference item (0.702 mg a.i./kg) | 17.4 | 25.8 | 23.4 | 13.4 | 16.5 | 13.5 | 11.1 | 14.3 | 13.4 | 6.7 | **16.2** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 12.4 | 24.2 | 26.9 | 32.3 | 31.1 | 27.8 | 35.8 | 29.3 | 27.2 | 30.5 | **27.8** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | DD |
| Reference item (0.702 mg a.i./kg) | 0.017 | 0.023 | 0.021 | 0.013 | 0.016 | 0.015 | 0.014 | 0.017 | 0.022 | 0.02 | **0.018** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | DD |
| Reference item (0.702 mg a.i./kg) | 0.018 | 0.018 | 0.016 | 0.009 | 0.011 | 0.01 | 0.008 | 0.01 | 0.01 | 0.005 | **0.010** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.702 mg a.i./kg) | 0.017 | 0.04 | 0.061 | 0.074 | 0.09 | 0.105 | 0.119 | 0.136 | 0.158 | 0.178 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.702 mg a.i./kg) | 0.012 | 0.03 | 0.046 | 0.055 | 0.066 | 0.076 | 0.084 | 0.094 | 0.104 | 0.109 |

**TABLE 49 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 11**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Reference item (1 mg a.i./kg) | 0.0 | 0.0 | 36.7 | 80.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 52.7 | 46.2 | 265.1 | 44.2 | 49.5 | 44.2 | 51.6 | 41.2 | 44.2 | 278.3 | **91.7** |
| 50 % sucrose solution containing 5 % acetone | 113.4 | 84.8 | 111.2 | 140.7 | 90.6 | 71.7 | 91.1 | 83.3 | 49.1 | 36.1 | **87.2** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 22.0 | 33.5 | 43.2 | 39.1 | 39.9 | 35.7 | 36.4 | 40.3 | 45.0 | 31.3 | **36.6** |
| 1 % acetone | 29.7 | 33.6 | 53.3 | 40.0 | 36.6 | 39.7 | 56.7 | 29.0 | 46.6 | 42.2 | **40.7** |
| 3 % acetone | 32.4 | 29.3 | 47.4 | 29.8 | 48.0 | 34.6 | 44.1 | 36.9 | 33.7 | 35.7 | **37.2** |
| 5 % acetone | 30.7 | 33.0 | 48.6 | 39.4 | 44.0 | 35.6 | 40.0 | 31.4 | 33.9 | 32.9 | **37.0** |
| Reference item (1 mg a.i./kg) | 29.8 | 32.9 | 20.5 | 24.7 | - | - | - | - | - | - | |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 16.7 | 28.8 | 16.7 | 34.5 | 34.7 | 31.1 | 31.1 | 36.1 | 40.4 | 2.5 | **27.3** |
| 1 % acetone | 24.4 | 29.0 | 26.8 | 35.5 | 31.4 | 35.1 | 51.3 | 24.8 | 42.0 | 13.4 | **31.4** |
| 3 % acetone | 27.2 | 24.7 | 20.9 | 25.4 | 43.0 | 30.2 | 39.0 | 32.8 | 29.2 | 7.9 | **28.0** |
| 5 % acetone | 19.4 | 21.7 | 37.3 | 28.1 | 32.6 | 24.3 | 28.6 | 20.1 | 22.6 | 21.6 | **25.6** |
| Reference item (1 mg a.i./kg) | 24.5 | 28.3 | -6.0 | 17.5 | - | - | - | - | - | - | |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 19.4 | 21.7 | 37.3 | 28.1 | 32.6 | 24.3 | 28.6 | 20.1 | 22.6 | 21.6 | **25.6** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (1 mg a.i./kg) | 0.03 | 0.033 | 0.02 | 0.025 | 0.027 | - | - | - | - | - | **0.027** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (1 mg a.i./kg) | 0.028 | 0.028 | -0.006 | 0.018 | -0.004 | - | - | - | - | - | **0.010** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (1 mg a.i./kg) | 0.03 | 0.063 | 0.083 | 0.108 | 0.135 | 0.135 | 0.135 | 0.135 | 0.135 | 0.135 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (1 mg a.i./kg) | 0.025 | 0.053 | 0.047 | 0.065 | 0.061 | 0.061 | 0.061 | 0.061 | 0.061 | 0.061 |

**TABLE 50 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 12**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 10.0 |
| Reference item (1.16 mg a.i./kg) | 0.0 | 0.0 | 0.0 | 10.0 | 40.0 | 70.0 | 86.7 | 93.3 | 96.7 | 100.0 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 63.0 | 51.3 | 75.7 | 55.7 | 24.3 | 42.7 | 36.3 | 57.0 | 41.7 | 75.3 | **52.3** |
| 50 % sucrose solution containing 5 % acetone | 99.0 | 130.3 | 79.7 | 156.0 | 55.7 | 69.7 | 56.7 | 83.3 | 100.7 | 91.0 | **92.2** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 39.9 | 36.5 | 44.8 | 36.1 | 31.3 | 35.9 | 40.1 | 35.5 | 48.4 | 38.6 | **38.7** |
| 1 % acetone | 48.8 | 34.6 | 43.7 | 33.9 | 36.9 | 43.4 | 51.9 | 40.9 | 55.5 | 46.7 | **43.6** |
| 3 % acetone | 50.5 | 36.7 | 47.8 | 35.2 | 43.1 | 55.4 | 59.1 | 52.2 | 55.4 | 54.6 | **49.0** |
| 5 % acetone | 45.6 | 42.6 | 42.4 | 35.6 | 42.0 | 50.4 | 52.5 | 58.3 | 54.5 | 56.6 | **48.1** |
| Reference item (1.16 mg a.i./kg) | 43.2 | 31.7 | 25.9 | 24.8 | 29.3 | 26.6 | 37.8 | 26.7 | 32.0 | 89.0 | **33.6** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 35.4 | 32.8 | 39.3 | 32.1 | 29.5 | 32.8 | 37.5 | 31.4 | 45.4 | 33.1 | **34.9** |
| 1 % acetone | 42.5 | 29.5 | 36.1 | 28.3 | 34.5 | 39.1 | 48.3 | 35.2 | 51.3 | 39.2 | **38.4** |
| 3 % acetone | 44.2 | 31.6 | 40.2 | 29.6 | 40.7 | 51.1 | 55.5 | 46.5 | 51.2 | 47.1 | **43.8** |
| 5 % acetone | 39.3 | 37.5 | 34.8 | 30.0 | 39.6 | 46.1 | 48.9 | 52.6 | 50.2 | 48.8 | **42.8** |
| Reference item (1.16 mg a.i./kg) | 36.9 | 26.6 | 18.3 | 19.2 | 26.6 | 18.9 | 17.6 | 12.5 | 11.2 | 13.7 | **22.1** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 35.7 | 32.7 | 32.5 | 25.7 | 32.1 | 40.5 | 42.6 | 48.4 | 44.2 | 46.3 | **38.1** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (1.16 mg a.i./kg) | 0.05 | 0.037 | 0.03 | 0.029 | 0.034 | 0.031 | 0.044 | 0.031 | 0.037 | 0.103 | **0.039** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (1.16 mg a.i./kg) | 0.031 | 0.031 | 0.021 | 0.022 | 0.031 | 0.022 | 0.02 | 0.015 | 0.013 | 0.016 | **0.030** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (1.16 mg a.i./kg) | 0.05 | 0.087 | 0.117 | 0.146 | 0.18 | 0.211 | 0.255 | 0.286 | 0.323 | 0.426 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (1.16 mg a.i./kg) | 0.043 | 0.074 | 0.095 | 0.117 | 0.148 | 0.17 | 0.19 | 0.205 | 0.218 | 0.234 |

**TABLE 51 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 13**

**Mortality in %**

| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 6.7 |
| Reference item (0.9 mg a.i./kg) | 0.0 | 0.0 | 3.3 | 60.0 | 93.3 | 96.7 | 100.0 | 100.0 | 100.0 | 100.0 |

**Evaporation**

| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 50 % sucrose solution | 90.3 | 95.0 | 96.0 | 97.3 | 93.3 | 95.7 | 90.7 | 102.3 | 96.3 | 158.3 | **101.5** |
| 50 % sucrose solution containing 5 % acetone | 113.0 | 121.3 | 148.0 | 122.7 | 131.0 | 123.3 | 114.0 | 126.0 | 118.3 | 118.0 | **123.6** |

**Food consumption in mg/bee**

| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Untreated control | 34.2 | 28.7 | 41.3 | 34.9 | 37.1 | 37.5 | 38.8 | 30.6 | 35.7 | 37.8 | **35.7** |
| 1 % acetone | 32.8 | 33.0 | 25.6 | 26.7 | 32.0 | 33.3 | 33.1 | 37.4 | 32.0 | 31.9 | **31.8** |
| 3 % acetone | 27.6 | 36.4 | 31.1 | 30.3 | 31.2 | 30.2 | 29.0 | 23.1 | 33.8 | 29.5 | **30.2** |
| 5 % acetone | 30.2 | 25.6 | 37.3 | 34.4 | 32.9 | 22.1 | 29.2 | 31.0 | 25.8 | 28.4 | **29.7** |
| Reference item (0.9 mg a.i./kg) | 36.0 | 37.8 | 31.4 | 27.3 | 43.3 | 61.5 | 101.0 | - | - | - | **40.6** |

**Food consumption in mg/bee (corrected for evaporation; untreated)**

| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Untreated control | 25.2 | 19.2 | 31.7 | 25.2 | 27.8 | 27.9 | 29.7 | 20.4 | 26.1 | 22.0 | **25.5** |
| 1 % acetone | 23.8 | 23.5 | 16.0 | 17.0 | 22.7 | 23.7 | 24.0 | 27.2 | 22.4 | 16.1 | **21.7** |
| 3 % acetone | 18.6 | 26.9 | 21.5 | 20.6 | 21.9 | 20.6 | 19.9 | 12.9 | 24.2 | 13.7 | **20.1** |
| 5 % acetone | 18.9 | 14.3 | 26.0 | 23.1 | 20.1 | 10.2 | 16.9 | 18.7 | 13.4 | 16.6 | **17.8** |
| Reference item (0.9 mg a.i./kg) | 27.0 | 28.3 | 21.8 | 17.2 | 14.7 | 13.7 | 10.3 | - | - | - | **20.7** |

**Food consumption in mg/bee (corrected for evaporation; 5% acetone)**

| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 % acetone | 18.9 | 14.3 | 26.0 | 23.1 | 20.1 | 10.2 | 16.9 | 18.7 | 13.4 | 16.6 | **17.8** |

**Daily uptake of reference item µg a.i./bee/day**

| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | DD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Reference item (0.9 mg a.i./kg) | 0.032 | 0.034 | 0.028 | 0.025 | 0.039 | 0.055 | 0.091 | - | - | - | **0.037** |

**Daily uptake of reference item µg a.i./bee/day (corrected for evaporation)**

| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | DD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Reference item (0.9 mg a.i./kg) | 0.025 | 0.025 | 0.019 | 0.015 | 0.013 | 0.012 | 0.009 | - | - | - | **0.020** |

**Accumulated uptake of reference item µg a.i./bee**

| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Reference item (0.9 mg a.i./kg) | 0.032 | 0.066 | 0.094 | 0.119 | 0.158 | 0.213 | 0.304 | 0.304 | 0.304 | 0.304 |

**Accumulated uptake of reference item µg a.i./bee (corrected for evaporation)**

| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Reference item (0.9 mg a.i./kg) | 0.024 | 0.049 | 0.068 | 0.083 | 0.096 | 0.108 | 0.117 | 0.117 | 0.117 | 0.117 |

**TABLE 52 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 14**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 6.7 | 6.7 |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 |
| 3 % acetone | 3.3 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 13.3 |
| 5 % acetone | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 13.3 | 23.3 | 23.3 | 23.3 |
| Reference item (0.7 mg a.i./kg) | 0.0 | 0.0 | 0.0 | 13.3 | 13.3 | 23.3 | 50.0 | 56.7 | 73.3 | 83.3 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 72.0 | 72.3 | 99.3 | 107.7 | 83.0 | 69.3 | 79.3 | 73.7 | 82.3 | 75.7 | **81.5** |
| 50 % sucrose solution containing 5 % acetone | 111.0 | 74.3 | 52.0 | 79.0 | 90.3 | 72.3 | 67.0 | 48.3 | 84.7 | 73.0 | **75.2** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 29.4 | 25.9 | 43.9 | 41.5 | 44.0 | 64.0 | 32.0 | 57.1 | 42.7 | 57.1 | **38.4** |
| 1 % acetone | 19.5 | 35.1 | 33.8 | 32.8 | 34.4 | 30.8 | 23.5 | 43.0 | 51.5 | 55.6 | **37.5** |
| 3 % acetone | 21.4 | 33.6 | 34.8 | 35.3 | 26.1 | 27.2 | 27.5 | 47.4 | 47.1 | 33.0 | **33.7** |
| 5 % acetone | 32.6 | 34.5 | 26.6 | 41.0 | 36.1 | 48.4 | 50.4 | 49.4 | 40.0 | 51.0 | **36.3** |
| Reference item (0.7 mg a.i./kg) | 31.1 | 41.8 | 20.1 | 22.8 | 34.7 | 27.8 | 30.2 | 14.2 | 17.1 | 23.1 | **27.4** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 20.6 | 24.8 | 24.9 | 31.9 | 29.8 | 47.0 | 27.7 | 33.1 | 25.7 | 36.4 | **30.2** |
| 1 % acetone | 18.3 | 24.2 | 19.6 | 23.4 | 32.3 | 22.6 | 26.1 | 39.2 | 43.2 | 44.4 | **29.3** |
| 3 % acetone | 11.9 | 23.0 | 21.0 | 15.8 | 17.8 | 29.2 | 26.2 | 33.6 | 35.4 | 35.7 | **25.0** |
| 5 % acetone | 17.4 | 28.9 | 17.3 | 20.5 | 21.4 | 28.4 | 34.4 | 34.3 | 28.9 | 36.3 | **26.8** |
| Reference item (0.7 mg a.i./kg) | 23.9 | 34.6 | 10.2 | 12.0 | 24.6 | 19.4 | 18.0 | 4.0 | 2.3 | 13.7 | **17.5** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 13.5 | 27.8 | 18.1 | 24.4 | 21.8 | 29.0 | 36.4 | 35.3 | 25.1 | 31.6 | **26.3** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (0.7 mg a.i./kg) | 0.022 | 0.03 | 0.014 | 0.016 | 0.025 | 0.019 | 0.021 | 0.01 | 0.012 | 0.016 | **0.019** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (0.7 mg a.i./kg) | 0.024 | 0.024 | 0.007 | 0.008 | 0.017 | 0.013 | 0.013 | 0.003 | 0.002 | 0.01 | **0.010** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.7 mg a.i./kg) | 0.022 | 0.052 | 0.066 | 0.082 | 0.107 | 0.126 | 0.147 | 0.157 | 0.169 | 0.185 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.7 mg a.i./kg) | 0.017 | 0.041 | 0.048 | 0.056 | 0.073 | 0.086 | 0.099 | 0.102 | 0.104 | 0.114 |

**TABLE 53 Summary of the Test Results (Mortality, Evaporation, Food consumption, Corrected Food Consumption and Intake of Reference Item) Generated by Lab 15**

| | Mortality in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 |
| 3 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5 % acetone | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Reference item (0.9 mg a.i./kg) | 0.0 | 0.0 | 0.0 | 36.7 | 70.0 | 96.7 | 96.7 | 100.0 | 100.0 | 100.0 |

| | Evaporation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 50 % sucrose solution | 86.3 | 62.0 | 73.0 | 64.7 | 80.3 | 68.0 | 76.3 | 73.7 | 72.7 | 71.0 | **72.8** |
| 50 % sucrose solution containing 5 % acetone | 86.7 | 38.7 | 25.0 | 23.7 | 23.7 | 17.7 | 24.3 | 40.3 | 31.0 | 43.7 | **35.5** |

| | Food consumption in mg/bee | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 35.8 | 44.2 | 21.3 | 46.9 | 33.5 | 38.1 | 34.1 | 42.0 | 42.8 | 25.8 | **36.4** |
| 1 % acetone | 31.2 | 41.0 | 24.0 | 41.9 | 40.9 | 22.7 | 40.3 | 36.3 | 39.0 | 20.1 | **33.7** |
| 3 % acetone | 33.7 | 37.0 | 22.7 | 41.5 | 27.0 | 31.7 | 39.8 | 36.8 | 44.7 | 26.1 | **34.1** |
| 5 % acetone | 38.8 | 23.7 | 24.4 | 38.8 | 32.4 | 35.3 | 39.2 | 30.0 | 38.4 | 29.7 | **33.1** |
| Reference item (0.9 mg a.i./kg) | 41.8 | 30.7 | 22.5 | 23.1 | 34.9 | 41.2 | 69.0 | 72.0 | - | - | **36.2** |

| | Food consumption in mg/bee (corrected for evaporation; untreated) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| Untreated control | 27.2 | 38.0 | 14.0 | 40.4 | 25.5 | 31.3 | 26.5 | 34.6 | 35.5 | 18.7 | **29.2** |
| 1 % acetone | 22.6 | 34.8 | 16.7 | 35.4 | 32.9 | 15.6 | 32.4 | 28.6 | 31.5 | 12.7 | **26.3** |
| 3 % acetone | 25.1 | 30.8 | 15.4 | 35.0 | 19.0 | 24.9 | 32.2 | 29.4 | 37.4 | 19.0 | **26.8** |
| 5 % acetone | 30.2 | 17.5 | 17.1 | 32.3 | 24.4 | 28.5 | 31.6 | 22.6 | 31.1 | 22.6 | **25.8** |
| Reference item (0.9 mg a.i./kg) | 33.2 | 24.5 | 15.2 | 16.6 | 21.1 | 16.6 | -7.3 | -1.7 | - | - | **18.6** |

| | Food consumption in mg/bee (corrected for evaporation; 5% acetone) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | $\bar{x}$ |
| 5 % acetone | 30.1 | 15.0 | 15.7 | 30.1 | 23.7 | 26.6 | 30.5 | 21.3 | 29.7 | 21.0 | **24.4** |

| | Daily uptake of reference item µg a.i./bee/day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (0.9 mg a.i./kg) | 0.038 | 0.028 | 0.02 | 0.021 | 0.032 | 0.037 | 0.062 | 0.065 | - | - | **0.033** |

| | Daily uptake of reference item µg a.i./bee/day (corrected for evaporation) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 | **DD** |
| Reference item (0.9 mg a.i./kg) | 0.022 | 0.022 | 0.014 | 0.015 | 0.019 | 0.015 | -0.007 | -0.002 | - | - | **0.020** |

| | Accumulated uptake of reference item µg a.i./bee | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.9 mg a.i./kg) | 0.038 | 0.066 | 0.086 | 0.107 | 0.139 | 0.176 | 0.238 | 0.303 | 0.303 | 0.303 |

| | Accumulated uptake of reference item µg a.i./bee (corrected for evaporation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d1 | d2 | d3 | d4 | d5 | d6 | d7 | d8 | d9 | d10 |
| Reference item (0.9 mg a.i./kg) | 0.03 | 0.052 | 0.066 | 0.081 | 0.1 | 0.115 | 0.108 | 0.106 | 0.106 | 0.106 |