Final Report

Tunnel Test: Assessment of Side Effects of Confidor SL 200 on the Honey Bee (*Apis mellifera* L.) in Apple Orchard Following Application before flowering (Mouse-Ear Stage) of the Crop

Data Requirements

Based on EPPO Guideline No. 170

Study Director

[redacted]

Date

09NOV2001

Testing Facility

Arbeitsgemeinschaft
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IFU Umweltanalytik GmbH
Eutinger Str. 24
D-75223 Niefern-Öschelbronn
Germany

Sponsor

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D-51368 Leverkusen
Germany

Study Identification Code

Test substance: Confidor SL 200
Study code: 20011099/01-BZEU

Statement of Confidentiality

Statement of Compliance with the Principles of Good Laboratory Practice

The study described in this report was conducted in compliance with the most recent edition of:

- The Principles of Good Laboratory Practice (GLP), Chemikaliengesetz, attachment 1, Germany.
- The OECD Principles of Good Laboratory Practice.

The German requirements are based on the OECD Principles of Good Laboratory Practice which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF and MITI) on the base of intergovernmental agreements.

- The weather data (temperature and precipitation) were not recorded under GLP

[redacted]

Statement of Quality Assurance Unit

[redacted]

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1 Summary

Report: [redacted]: Tunnel Test: Assessment of Side Effects of Confidor SL 200 on the Honey Bee (*Apis mellifera* L.) in Apple Orchard Following Application before flowering (Mouse-Ear Stage) of the Crop

Source: GAB, unpublished report No: 20011099/01-BZEU, (09NOV2001).

Guidelines: Based on EPPO Guideline No. 170

Deviations: No major deviations.

GLP: yes (certified laboratory).

Materials and Methods

Test substance: Name: Confidor SL 200;
purity: 194 g/L (nominal: 200 g/L)

The following study was designed to determine the effects of Confidor SL 200 on the honey bee (*Apis mellifera* L.) under semi-field conditions in an apple orchard. The study was carried out in Germany near Karlsruhe at the test location Augustenberg. The test substance Confidor SL 200 was tested at an application rate of 0.105 kg a.s./ha in 500 L water/ha (amount of water was adapted to the tree height). The application was performed at the mouse-ear stage of the apple trees (BBCH-code 10, 30MAR2001). Untreated orchard plots with apple trees served as control.

This GLP compliant study was conducted based on the guideline of the European and Mediterranean Plant Protection Organisation No. 170 (EPPO, 1992).

After the application of the test substance before the start of flowering (04APR2001) 3 tunnel tents for the test substance treatment were build up over the treated plots of apple trees. In the control 3 tunnels were set-up over untreated plots of apple trees from the same variety. At the start of full flowering (23APR2001) one small bee colony was placed in each tunnel of the test substance treated apple plots and the untreated apple plots for the control.

Mortality, foraging activity, behaviour, and condition of the colonies and the development of the bee brood were assessed over a period of 7 days.

The influence of the test substance Confidor SL 200 was evaluated by comparing the bees in the pesticide-treated tunnels to those in the control tunnels regarding the following observations:

- Mortality at the edge of the treated area and in the bee traps.
- Flight intensity in the crop (number of flying bees/tree/minute).
- Flight intensity in front of the hives (number of bees leaving/entering the hive/minute).
- Behaviour of the bees on the crop and around the hive.
- Development of the bee brood.

Dates of work: 30MAR2001 - 30APR2001

Findings

Effects on honey bee mortality:

No increased number of dead bees in the dead bee traps and on the linen at the edge of the treated area could be noticed in the test substance treatment in comparison to the control. The daily average of dead bees in the dead bee trap was 4.8 dead bees/tent in the test substance treatment and 7.3 dead bees/tent in the control. During evaluation day 1 – 7 the daily average of dead bees recorded on the linen was 7.9 dead bees/tent in the test substance treatment compared to 12.4 dead bees/tent in the control. The total daily average of dead bees per tent was 12.7 dead bees/tent in the test substance treatment and 19.7 dead bees/tent in the control.

Effects on honey bee flight intensity:

During the 7 days of assessments the daily average flight intensity in the crop ranged from 0.04 - 20.89 forager bees/tree/minute/tent in the test substance treatment and from 0.00 – 20.22 forager bees/tree/minute/tent in the control. The overall daily average of flight intensity on the apple trees during the period of assessments was similar in both treatments with 10.05 forager bees/tree/minute/tent in the test substance treatment compared to 9.24 forager bees/tree/minute/tent in the control.

The daily average number of forager bees leaving/entering the hive per minute was 10.31 bees/tent in the test substance treatment and 10.47 bees/tent in the control during the period of assessments.

Conditions of the colonies and effects on honey bee brood development:

The conditions of the colonies and the bee brood development showed no abnormal difference which could be attributed to the influence of the test substance.

Effects on behaviour of the bees:

No abnormal difference in behaviour of the bees was observed between the test substance treatments and the control treatments at any time during the period of assessment.

Conclusion:

The treatment of apple trees at the mouse-ear stage with Confidor SL 200 at an test rate of 0.105 kg a.s./ha in 500 L water/ha did not cause adverse effects to honey bee mortality, flight intensity in the crop or the brood development of the colonies in this semi-field study.

2 Time Schedule

Study initiation date:	29MAR2001
Start of the experimental phase:	30MAR2001
End of the experimental phase:	30APR2001
Draft report:	17OCT2001
Study completion date:	09NOV2001

3 Personnel

Study Director:

Technical personnel involved: [redacted]

4 Study Objective

The following study was designed to determine the effects of Confidor SL 200 on the honey bee (*Apis mellifera* L.) under semi-field conditions in an apple orchard. The study was carried out in Germany near Karlsruhe at the test location Augustenberg. The test substance Confidor SL 200 was tested with an application rate of 0.105 kg a.s./ha in 500 L water/ha. The application was performed at the mouse-ear stage of the apple trees (BBCH-code 10).
This GLP compliant study was conducted based on the guideline of the European and Mediterranean Plant Protection Organisation No. 170 (EPPO, 1992).

5 Material and Methods

5.1 Test Substance

Name:	Confidor SL 200
GAB-Code:	20011099
Batch number:	233925888
Formulation type:	SL
Active ingredient:	imidacloprid
CAS No.:	138261-41-3
Amount of a.s. (analysed):	194 g/L
Amount of a.s. (nominal):	200 g/L
Density:	1.121 g/mL
Appearance / colour:	liquid / transparent bright brown
Date of analysis:	04SEP2000
Expiry date:	04SEP2001
Storage conditions:	dry and dark, temperature from 0 °C – 40 °C
Safety symbol:	Xn, N
Intended Use (Target(s):	insecticide
Application rate in this study:	0.105 kg a.s./ha in 500 L water/ha

Purity and Composition

All necessary specifications of purity and composition of the test substance were provided by the sponsor.

Stability and Homogeneity in the Spraying Solution

The test substance was diluted under field conditions and applied immediately afterwards onto the plant surface. The stability under test conditions was therefore of no relevance for this type of experiment.

5.2 Control

The control plots were untreated.

5.3 Dosage / Concentration

The application rate(s) of the test substance used in this study are presented in Table 1.

Table 1: Details about application rates

Treatment	Application rate per ha	Water rate to apply [L/ha]
Test substance	0.105 kg a.s.	500
Control	--	--

5.4 Test Organism

As test organism the honey bee, *Apis mellifera carnica* (Hymenoptera, Apoidea) was used.

The honey bee is an important beneficial insect due to its pollination activity in fruit, berry and seed growing. Due to the specific use of honey bees in the crops to be pollinated (migratory beekeeping) they are an irreplaceable productive factor. In addition to this, they contribute to the preservation of a multitude of wild flowering plants because of their high constancy in flower pollination activity.

5.5 Principles of the Study

The test substance Confidor 200 SL was applied at an application rate of 0.105 kg a.s./ha in 500 L water/ha at the mouse-ear stage of the apple trees (BBCH-code 10). Untreated orchard plots with apple trees served as control.

The tunnels (3 replicates per treatment) were installed after the application of the test substance before the full flowering of the apple trees.

The effect of the test substance was examined on small bee colonies under a net placed over each plot with flowering apples by assessing the mortality, foraging activity and condition of the colonies during the flowering period over 7 days by comparing the results of the test substance plots to the results found in the control bees held under identical conditions.

No insecticides or plant protection products with side-effects on bees were used during the exposure phase of the bees.

5.6 Description of the Test Method

5.6.1 Test Location, Test Plant and Test Tents

The semi-field test was located in Southern Germany near Karlsruhe at the location Augustenberg (see Figure 1 and Table 2).

The crop used was apple of the same variety (details are summarised in Table 3). The size of each tunnel was at 9 – 12 apple trees and the distance between each tunnel was at least 5 trees (in the row). All trees of one tunnel were covered with a net. 10 meters of untreated trees were left uncovered as an barrier between the test substance and the control tunnels.

Figure 1: Location of the test site in Germany

Table 2: Description of the test site

Location	Augustenberg
Zip code	76227
Region	Baden-Württemberg
Country	Germany
Meters above sea level	150
Slope	2 %

Table 3: Description of the crop at the test site

Crop	Apple
Variety	Cadel
Date of planting	Spring 1995
Distance between rows	3.5 m
Distance between trees in the row	1.0 m
Height of trees at start of bee testing	2.40 – 2.60

The size of each tunnel (covered trees) was 10 m long, 5 m wide and 3.5 m high. The edges of each plot was covered with linen (0.6 m) of the same width all around (see Figure 2).

One hive per tunnel was introduced in the tents in the evening at start of flowering of the apple trees (23APR2001). In front of the bee hives, bee traps were installed to determine the number of dead bees at the entrance.

A half-full bucket of water was placed into each plot. The surface of the water was covered with scraps of polystyrene to prevent the bees from drowning.

The arrangement of the test tunnels is given in Figure 3.

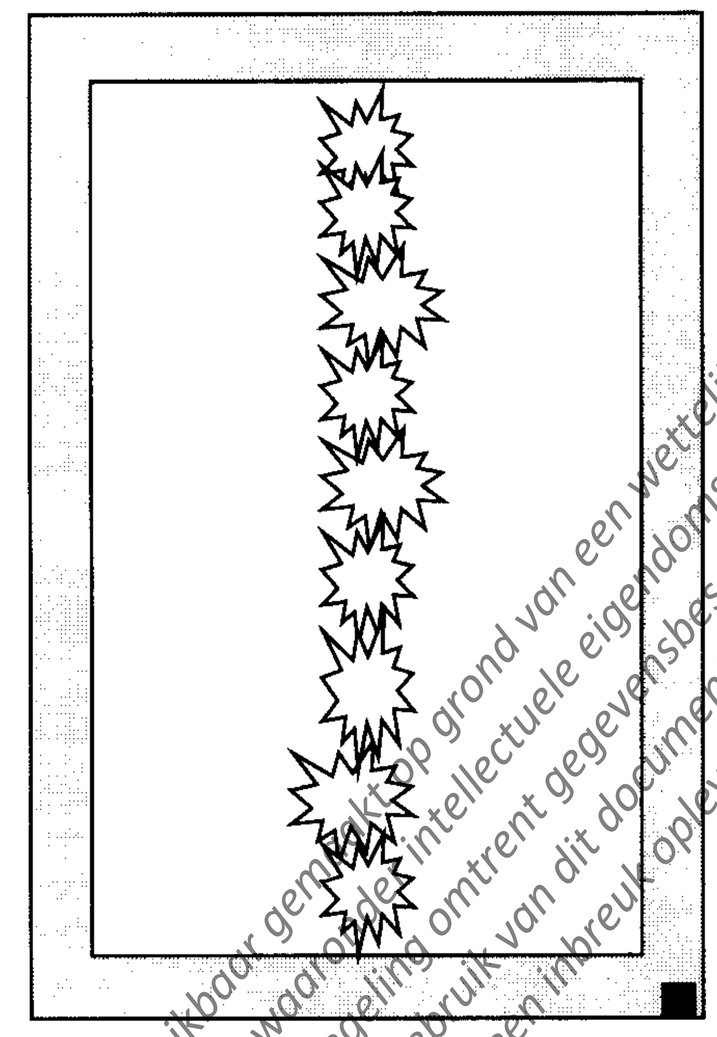
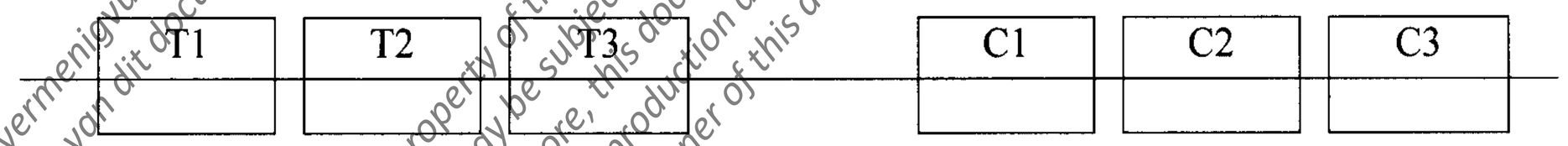


Figure 2: Location of the colony and the linen sheets in the tent



= Test tents used for bee testing (3 tents per treatment; T1 – T3 : Test substance tents; C1 – C3: Control tents)

= Espalier of apples

Figure 3: Sketch of the arrangement of the tents of the treatments

5.6.2 Design and Lay-out of the Test

The semi-field test was carried out once with the following treatments and replicates:

Table 4: Test design

Treatment	**Replicates**
Test substance	3 tents (plots) with 1 colony each
Control	3 tents (plots) with 1 colony each

5.6.3 Experimental Bee Colonies

For the test, small healthy colonies with 5 combs (size of the combs ("Zandermaß": 420 mm x 220 mm) and approximately 8.000 worker bees per colony were used. All nuclei were produced at the same time. The corresponding queens originated from one breeding line in order to guarantee uniform bee material in all treatments.

Furthermore the following criteria for the nuclei were met:

- at least two brood combs containing eggs, larvae and capped cells
- at least one honey and pollen comb
- bees were free of *Nosema* disease symptoms and other bee diseases

Wooden bee traps (35 cm x 35 cm) with gauze on bottom and on 50 % of the top were attached to the entrance of the hives in order to register those dead bees which were carried out of the hives.

The hives were introduced into the tents in the evening at the start of flowering of the apple trees at the same time for all tents.

5.6.4 Application of the Test Substance

The application of the spray suspension was carried out with a spraying equipment used for commercial applications. An exact description of the equipment is given in Table 5.

The test substance was weighed out in the laboratory and then transported to the test site. Transport conditions were recorded. The appropriate amount of spray solution was prepared immediately before application.
The actual applied amount/volume of dilution was determined after the treatment and was recorded in the raw data (see Table 5).

The following conditions were met for application of the test substance:

- Mouse-ear stage of the apple trees (BBCH-code 10)
- Wind speed was below 2 m/sec.

Details of the conditions during the application are given in Table 5.

Table 5: Details of treatment

		Test Substance
Date		30MAR2001
Time	[h:min]	8:50 – 8:56
Device		
Sprayer		Sprayer mounted on a tractor
Trade name of sprayer		Meyers
Nozzles (No./type)		10 / Albuz ATR red
Pressure	[bar]	9.5
Application speed	[km/h]	6.0
Technical faults		no
Dosage		
Plot size	[m²]	210.0
Active ingredient/ha	[kg]	0.105
Water volume/ha	[L]	500
Active ingredient/ha (actually applied)	[kg]	0.115
Water volume/ha (actually applied)	[L]	547.62
Environment/Crop		
Temperature	[°C]	7.0 – 7.3
Humidity	[%]	76 - 79
Wind speed	[m/s]	1.0
Rainfall that day/next day	[mm]	0.9*/0.0
Clouding	[%]	100
Target area conditions		Dry
Distance to target area	[cm]	20 - 30

* Remark: No rainfall from the end of the application until 6:00 p.m. in the evening

5.6.5 Recording of the Meteorological Data

During the test period, the following climatic data were recorded:

- temperature
- rainfall
- degree of cloud formation (estimated at time of evaluation)
- wind speed (only during application).

The meteorological data (temperature and rainfall) were recorded approximately 9 km from the test site Augustenberg by a weather station in Karlsruhe (Meteo-Media-Messnetz).

5.7 Mode of Assessment

5.7.1 Mortality of Adult Honey Bees

Bee traps with gauze on bottom and on 50 % of the top were attached to the entrance of the hives in order to register those dead bees which are carried out of the hives. Furthermore, the mortality was recorded in the crop. Waterpermeable linen sheets (area 16.5 m^2) were spread out at the edge of the tunnels to count the number of dead bees in the plots (see Figure 2).

The observations of mortality were carried out according to the scheme given in Table 6.

Table 6: Evaluation of mortality

Time of the test	ED	Evaluation of mortality*	
		Test substance treatment	Control treatment
1st to 7th day after the set-up of the hives in the tunnels	1, 2,....7	Once a day at about the same time of day	

Remark: * At each assessment day the number of dead bees were counted and removed.
ED = Evaluation days

5.7.2 Flight Intensity

The observations of the flight intensity in the tunnels, which started one day after the bee hives were set-up, took place in three marked trees distributed over the plot of flowering apple trees per tent. At each assessment time the number of bees that were both foraging on the marked trees and flying over the tree were counted for one minute. At each assessment time bees leaving/entering the hive were counted

(bees/minute). The observations were carried out according to the scheme given in Table 7.

Table 7: Evaluation of flight intensity

Time of the test	ED	Evaluation of flight intensity	
		Test substance treatment	Control treatment
1st and 2nd day after the set-up of the test colonies	1 and 2	Three times a day at high flight activity of the bees	
3rd and 4th day after the set-up of the test colonies	3 and 4	Two times a day at high flight activity of the bees	
5th to 7th day after the set-up of the test colonies	5,...7	Once a day at about the same time of day at high flight activity of the bees	

Remark: ED = Evaluation days

The assessments was performed approx. at the same time in both test groups.

5.7.3 Conditions of the Colonies, Development of the Bee Brood

The condition of the colonies was recorded and the development of the bee brood was checked on the day of set-up of the test colonies in tents and 7 days after the set-up of the colonies in the orchard.

In order to record effects of the test substance, the following parameters were assessed:

- Strength of the colony (number of combs covered with bees)
- Presence of a healthy queen
- Estimate of the pollen storage area and area with nectar
- Estimate of the area containing eggs, larvae and capped cells.

At each assessment both sides of one comb were assigned to be 100 % and the percentage area covered with the brood stages, pollen and nectar on the comb was estimated. This was done for all combs per hive. The assessments were performed by experienced personnel. Afterwards the mean values were calculated for each hive and assessment date. The mean values (%) for the brood stages (eggs, larvae and pupae) per hive were calculated based on the total number of combs cover with brood. The amount of eggs, larvae and capped brood was given in percent of total brood population for each type of brood (see Table 16 and Table 17 in the Appendix A2).

5.7.4 Observations at the Entrance of the Hives

In addition to the assessments of mortality and flight intensity, the behaviour of the bees returning to the entrance of the hives and during foraging in the crop was observed on the days after the set-up of the colonies in the tents once a day.

5.7.5 Flowering Stage of the Crop and Fruit Buds

The stage of blooming of the apple trees was recorded on each day of assessment (evaluation day 1 – 7) and was documented in the raw data (see Table 18 in the Appendix A2).

The assessment of the fruit buds in the different test groups in the tents was not performed since the pollination of the apple flowers by bees in tents is spoiled by the conditions.

5.8 Evaluation of the Test Results

The influence of the test substance Confidor SL 200 was evaluated by comparing the bees of the test substance treatment with the bees of the control hives in view of the following observations:

- Mortality in front of the bee hives and in the edge of the tunnels
- Flight intensity on the crop and in front of the hives
- Development of the bee brood
- Behaviour of the bees at the entrance of the hives

Differences between treatments are usually evaluated one sided higher for mortality data compared to the control. Since the mortality data (dead bee trap and linen sheets) in the test substance treatment was on the same or lower level compared to the control treatment no statistical evaluation of the results was carried out, because of no significant increased mortality in the test substance treatment compared to the control treatment.

6 Deviations from the Study Plan

The study was performed according to the study plan dated 29MAR2001 and with the following deviation:

1. Flowering Stage of the Crop and Fruit Buds (item 2.7.5 of the study plan)

   Deviation: The stage of blooming was recorded on each day of assessments.
   The fruit buds were not assessed.

   Reason: To get exact data and differences between the tents regarding the flowering of the trees on each evaluation day.
   Since the pollination of the apple flowers by bees in tents is spoiled by the conditions it is not possible to compare different treatments. Therefore the fruit buds of the apple trees in tents were not assessed.

   Effect on the study: More data about the flowering stages available.
   No data for fruit buds evaluation of the treatments available.

2. Conditions of the Colonies, Development of the Bee Brood (item 2.7.3 of the study plan)

   Deviation: The first brood assessment was carried out on the day of the set-up of the colonies in tents instead of 2 ± 1 days before the set-up as stated in the study plan.

   Reason: Organizational reason.

   Effect on the study: None, because it had no impact on the results of the trial.

The report reflects the conduct of the study.

7 Results

7.1 Dates of the Test

The important dates of the test are given in Table 8.

Table 8: Dates of the test

Activity	Date
Application of the test substance	30MAR01
Installing of the tents	04APR01
Brood assessment before introduction of hives in tents	23APR01
Set-up of the hives in tents	23APR01
Start evaluation of mortality and flight intensity	24APR01
Last evaluation of mortality and flight intensity	30APR01
Last brood assessment	30APR01

7.2 Mortality

Dead bees in the dead bee trap

Figure 4 shows the average mortality in the dead bee trap of the tents of the test substance treatment and the control (see also Table 10 and Table 11 in the Appendix A2). The period of evaluations is identified by day 1 – 7 after introduction of the hives in tents.

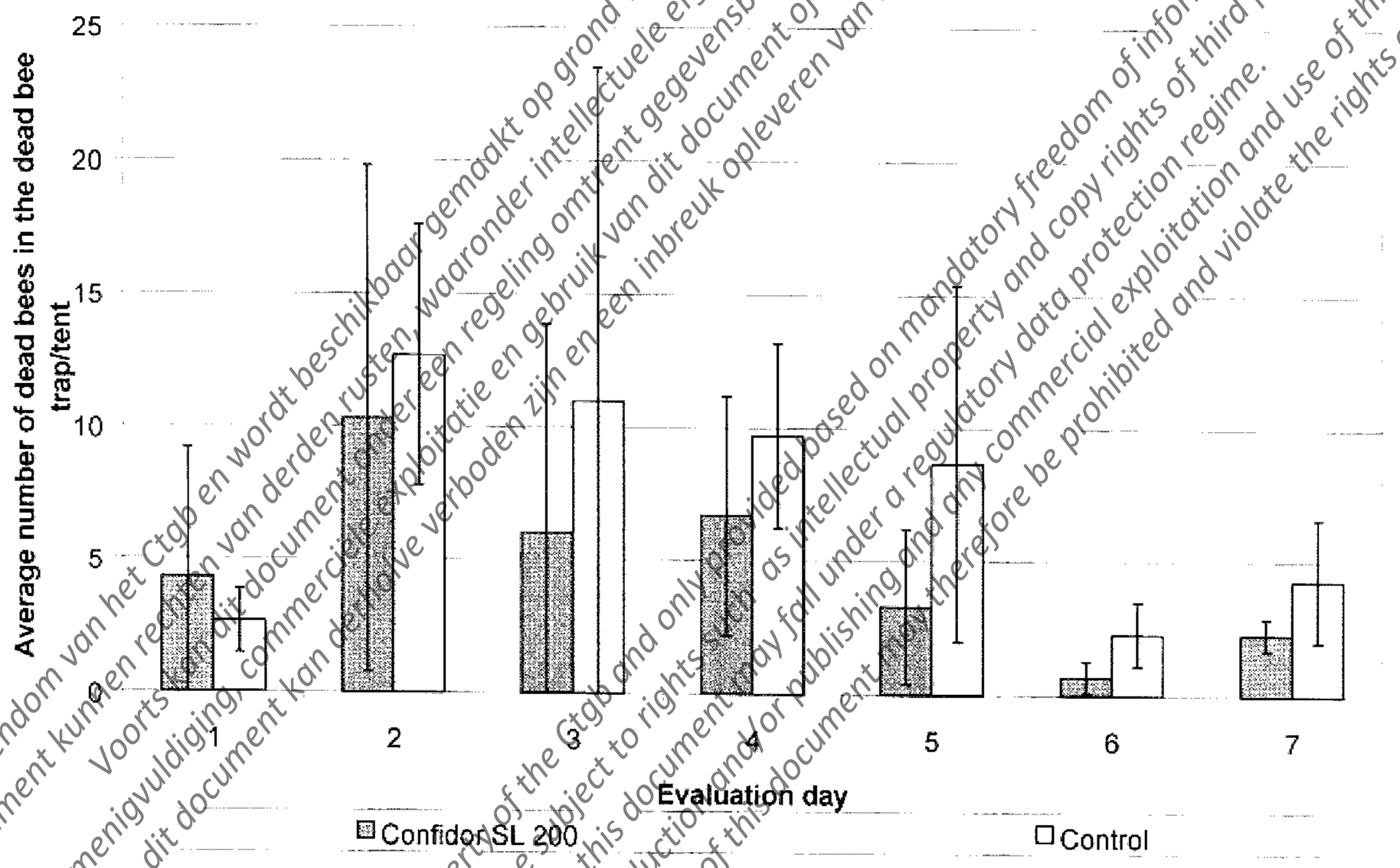


Figure 4: Average number of dead bees/tent/day collected in the dead bee traps in the test substance treatment and the control on the evaluation days 1 – 7 after start of bee exposure

During the entire test period the average number of dead bees in the dead bee trap of the test substance treatment was similar or lower compared to the control (see Figure 4). The mortality in the dead bee trap ranged from 0.7 – 10.3 dead bees/tent/day in the test substance treatment and 2.3 – 12.7 dead bees/tent/day in the control group from evaluation day 1 to 7 (see Table 10 and Table 11 in the Appendix A2). The daily average number of dead bees in the dead bee trap was 4.8 dead bees/tent in the test substance treatment compared to 7.3 dead bees/tent in the control (see Table 10 and Table 11 in the Appendix A2).

Dead bees on the linen sheet

Figure 5 shows the average mortality on the linen sheet per tent of the test substance treatment and the control (see also Table 10 and Table 11 in the Appendix A2).

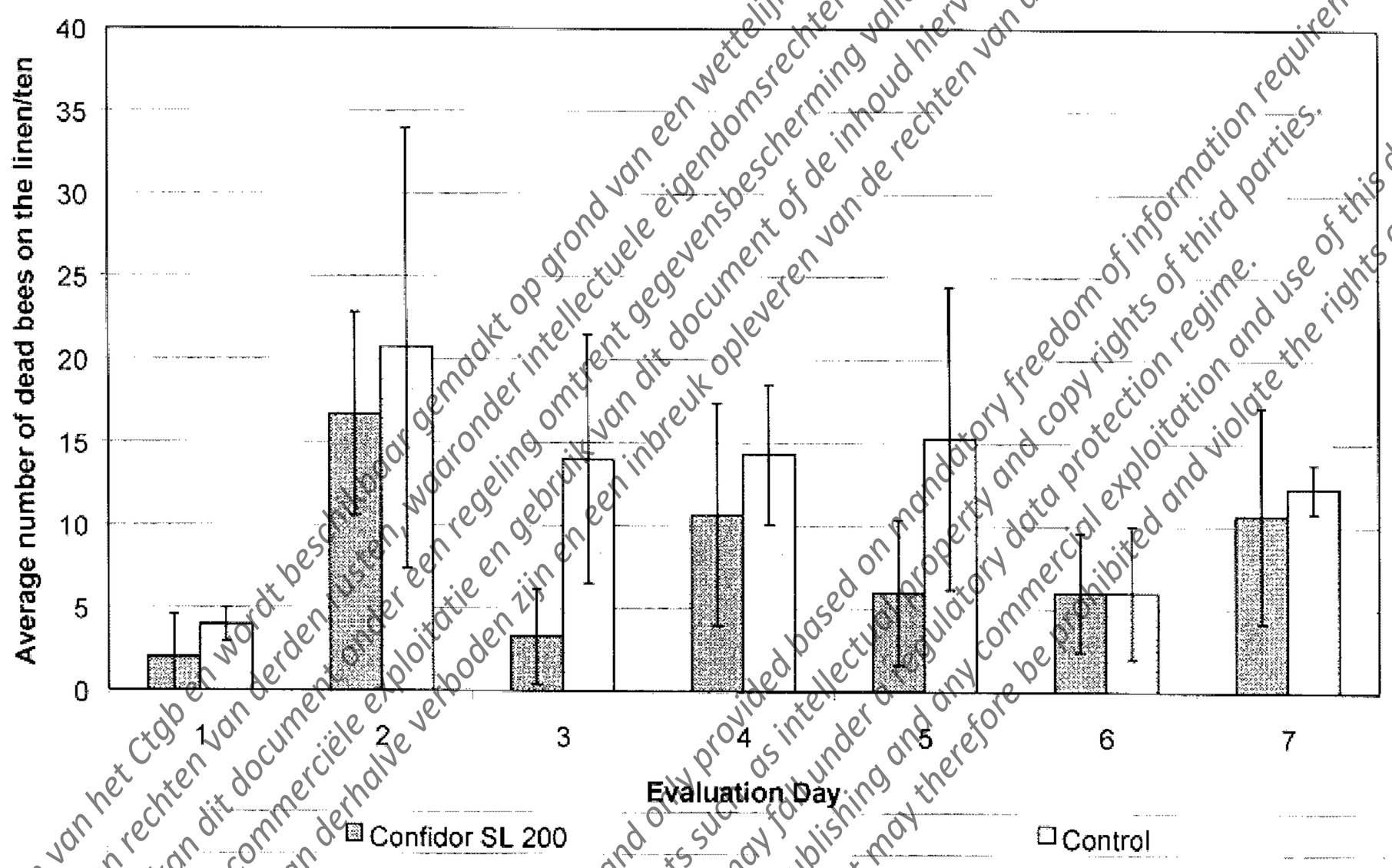


Figure 5: Average number of dead bees/tent/day collected on the linen sheet in the test substance treatment and the control on the evaluation days 1 – 7 after start of bee exposure

In both treatments the mean number of dead bees on the linen/tent was on a low level during the entire test period of 7 days, but was higher in the control on almost every assessment day than in the test substance treatment (see Figure 5). During evaluation day 1 – 7 the daily average of dead bees recorded on the linen was 7.9 dead bees/tent in the test substance treatment compared to 12.4 dead bees/tent in the control (see Table 10 and Table 11 in the Appendix A2).

Total number of dead bees

Figure 6 shows the average mortality per tent (dead bee trap and on the linen sheet) of the test substance treatment and the control (see also Table 10 and Table 11 in the Appendix A2).

The total number of dead bees per tent was on a low level in both treatments on the first evaluation day but increased on the following assessment day (see Figure 6). The increase of mortality might be caused by the transport of the hives in tents and by the adaptation of the forager bees to the conditions in the tents. However, the average number of dead bees/tent/day was in the biological range of bee mortality normally occurs under semi-field condition in both treatments during the evaluation period of 7 days. The daily average number of dead bees per tent was 12.7 dead bees/tent in the test substance treatment compared to 19.7 dead bees/tent in the control (see Table 10 and Table 11 in the Appendix A2).

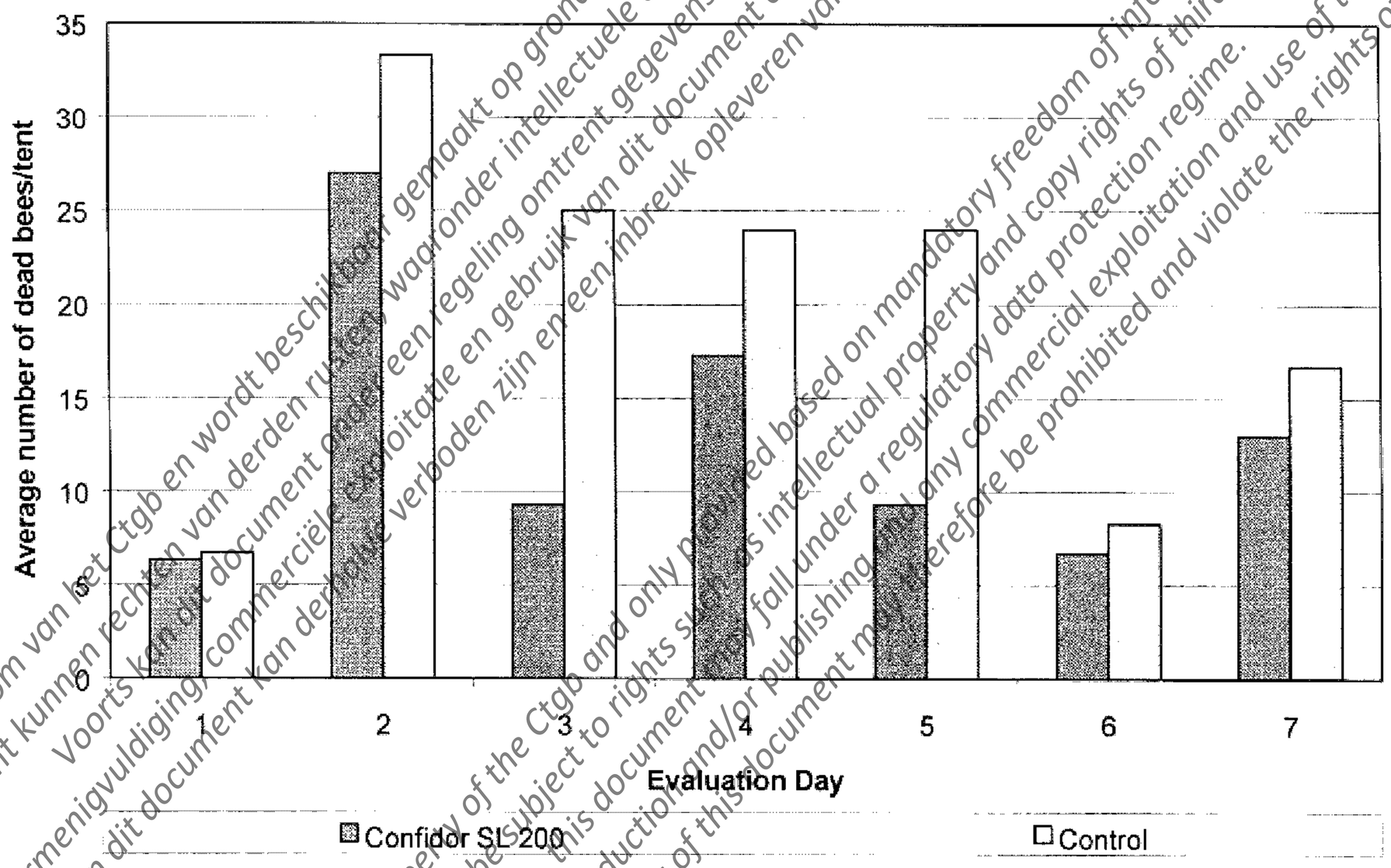


Figure 6: Average number of dead bees/tent/day collected in the tents (dead bee trap and on the linen sheet) in the test substance treatment and in the control on the evaluation days 1 – 7 after start of bee exposure

7.3 Flight Intensity

In the crop

Figure 7 shows the daily average flight intensity on the apple trees per tent and minute of the test substance treatment and the control (for data of single evaluations

see also Table 12 and Table 13 in the Appendix A2). The period of evaluations is identified by day 1 – 7 after introduction of the hives in tents.

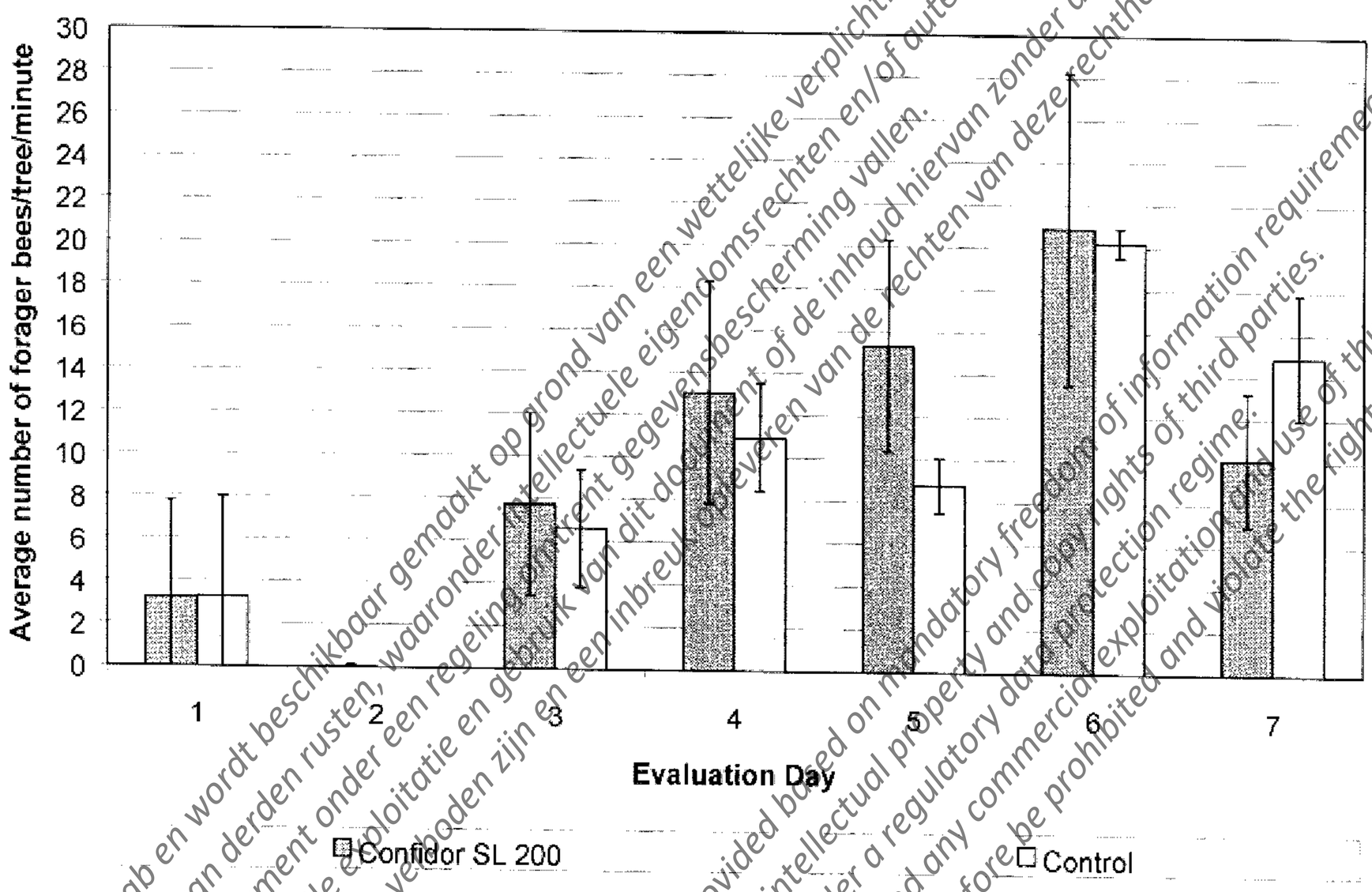


Figure 7: Average number of forager bees per tree and minute (test substance treatment and the control) on the evaluation days 1 – 7 after start of bee exposure

On the first day of assessments the mean flight intensity was similar in both treatments (see Table 12 and Table 13 in the Appendix A2). Since on evaluation day 2 rainfall occurred at the test site, the average flight intensity was decreased in the test substance treatment and no forager bees were noticed in the control (see Table 12 and Table 13 in the Appendix A2). On the following days (ED 3 – 7) the average flight intensity showed a tendency to increase over the assessment period. This might be caused by the adaptation of the forager bees to the conditions in the tents and the increase of open flowers during the period of assessments in both treatments (see Figure 7). During the 7 days of assessments the daily average flight intensity ranged from 0.04 - 20.89 forager bees/tree/minute/tent in the test substance treatment and from 0.00 - 20.22 forager bees/tree/minute/tent in the control (see Table 12 and Table 13 in the Appendix A2). The overall daily average of flight intensity during the period of assessments was similar in both treatments with 10.05 forager

bees/tree/minute/tent in the test substance treatment compared to 9.24 forager bees/tree/minute/tent in the control (see Table 12 and Table 13 in the Appendix A2).

In front of the hive

Figure 8 shows the daily average flight intensity in front of the hives (number of bees leaving/entering the hive per minute) of the test substance treatment and the control (for data of single evaluations see also Table 14 and Table 15 in the Appendix A2).

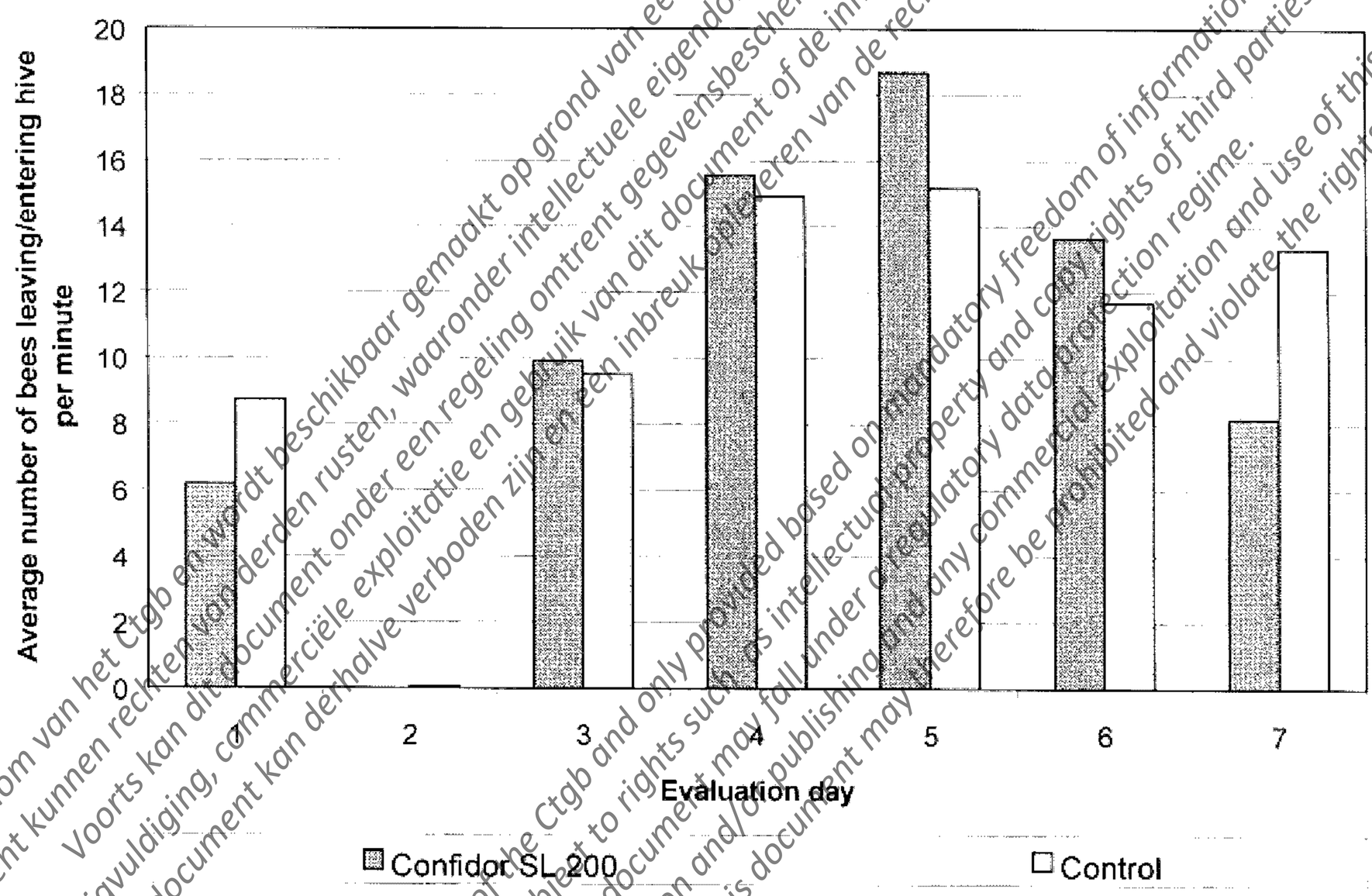


Figure 8: Average number of forager bees leaving/entering the hive per minute (test substance treatment and the control) on the evaluation days 1 – 7 after start of bee exposure

The colonies of test substance and the control treatment showed a similar average activity of forager bees leaving/entering the hive per minute on the assessment days 1 - 6 (see Figure 8). On the last assessment day the flight activity in front of the hives was increased in the control compared to the test substance treatment. The daily average number of forager bees leaving/entering the hive per minute was 10.31 bees/tent in the test substance treatment and 10.47 bees/tent in the control during the period of assessments (see Table 14 and Table 15 in the Appendix A2).

7.4 Bee Brood

During the observation period changes and fluctuations in the relative amount of the different pre-imaginal stages, i.e. egg stage, larval and pupal stage, occurred in almost every colony of the test substance groups and the control group (see Table 16 and Table 17 in Appendix A2).
At the brood assessment before bee exposure as well as on the brood assessment carried out 7 days after the introduction of bees in tents all brood stages were present in the colonies of both, the test substance and the control treatment (see Table 16 and Table 17 in the Appendix A2). The continued presence of eggs, larvae and pupae in the colonies showed that the queens survived and the colonies were in a good condition after the end of exposure. The strength of the colonies was stable or increased during the exposure period in both treatments compared to the assessment carried out before the start of bee exposure.

7.5 Behaviour of the Bees

No abnormal difference in behaviour of the bees was observed between the test substance treatment and the control any time during the period of assessments.

8 Conclusions

The semi-field study was carried out following the EPPO guideline No. 170: Guideline on test methods for evaluation the side-effects of plant protection products on honey bees (EPPO, 1992). The test substance Confidor SL 200 was tested at an application rate of 0.105 kg a.s./ha in 500 L water/ha. The application was performed at the mouse-ear stage of the apple trees (BBCH-code 10, 30MAR2001). Untreated orchard plots with apple trees served as control. After the application of the test substance at the start of flowering (04APR2001) 3 tunnel tents were build up over the treated/untreated plots of apple trees per treatment. At the start of flowering (23APR2001) one small bee colony was placed in each tunnel of the test substance treated apple plots and untreated apple plots for the control.

The average number of dead bees/tent/day was in the biological range of bee mortality normally occurs under semi-field condition in both, the test substance and the control treatment during the evaluation period of 7 days. The daily average of the number of dead bees per tent was 12.7 dead bees/tent in the test substance treatment compared to 19.7 dead bees/tent in the control.

The overall daily average of flight intensity in the crop during the period of assessments was similar in both treatments with 10.05 forager bees/tree/minute/tent in the test substance treatment compared to 9.24 forager bees/tree/minute/tent in the control.

At the brood assessment before bee exposure as well as on the brood assessment carried out 7 days after the introduction of bees in tents all brood stages were present in the colonies of both, the test substance and the control treatment. The continued presence of eggs, larvae and pupae in all colonies showed that the queens survived and the colonies were in a good condition after the end of exposure.

No abnormal difference in behaviour of the bees was observed between the test substance treatment and the control treatment at any time during the period of assessment.

The treatment of apple trees at the mouse-ear stage with Confidor SL 200 at an test rate of 0.105 kg a.s./ha in 500 L water/ha did not cause adverse effects to honey bee mortality, flight intensity in the crop or the brood development of the colonies in this semi-field study.

9 Archiving

For the periods demanded by the principles of GLP the following documents and materials will be archived:

- Study plan, raw data, comments of the sponsor on the draft report and final report.
- All data generated by the Quality Assurance Unit.
- A sample of the test substance.

All other materials and documents will be stored in the archives of the Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH. The premises for storing the documents and materials are settled according to the principles of Good Laboratory Practice in the organisation of the testing facility.

The study director / sponsor will receive the documents listed in the study plan.

10 References

EPPO (1992): Guideline in test methods for evaluating the side-effects of plant protection products on honey bees. - EPPO Bulletin **22**, 203 - 215.

11 Distribution

11.1 Study Plan

Original: Testing facility (1 x)
Sponsor (1 x)

11.2 Final Report

Original: Testing facility (1 x)
Sponsor (1 x)

11.3 Raw Data

Original: Testing facility (1x)

12 Appendix

A1 General Information

Table 9: Weather conditions during the trial; temperature and precipitation was recorded 9 km from the test site Augustenberg by a weather station in Karlsruhe (Meteo-Media-Messnetz)

Date	ED	Ø Temperature min/max [°C]	Precipitation [mm]	Cloud formation at time of evaluation [%]
30MAR2001	-	5.8 / 11.1	0.9	100
31MAR2001	-	3.4 / 15.9	0.0	-
01APR2001	-	2.2 / 20.1	0.0	-
02APR2001	-	5.3 / 23.1	0.0	-
03APR2001	-	11.8 / 15.1	0.0	-
04APR2001	-	6.2 / 18.0	5.0	-
05APR2001	-	5.0 / 12.8	1.1	-
06APR2001	-	6.9 / 14.5	2.5	-
07APR2001	-	9.4 / 13.1	0.0	-
08APR2001	-	2.6 / 15.4	6.7	-
09APR2001	-	6.2 / 9.8	2.3	-
10APR2001	-	8.1 / 12.5	5.7	-
11APR2001	-	6.8 / 13.5	0.2	-
12APR2001	-	4.9 / 12.2	0.0	-
13APR2001	-	1.4 / 7.4	0.0	-
14APR2001	-	-1.5 / 7.7	3.8	-
15APR2001	-	0.6 / 6.5	11.0	-
16APR2001	-	5.0 / 10.1	10.2	-
17APR2001	-	4.3 / 10.5	0.5	-
18APR2001	-	4.7 / 8.6	3.3	-
19APR2001	-	1.7 / 9.2	0.0	-
20APR2001	-	0.9 / 10.1	0.0	-
21APR2001	-	1.4 / 6.4	10.2	-
22APR2001	-	3.1 / 11.3	0.0	-
23APR2001	-	1.1 / 16.3	0.0	-
24APR2001	1	4.4 / 19.3	0.1	0 – 90
25APR2001	2	9.4 / 12.9	7.8	100
26APR2001	3	7.6 / 14.5	0.7	10 – 35
27APR2001	4	6.3 / 17.4	1.0	75 – 99
28APR2001	5	10.4 / 14.4	5.6	75 – 100
29APR2001	6	9.6 / 19.7	1.3	30 – 95
30APR2001	7	10.4 / 22.6	0.0	10 – 40

ED = Evaluation days after the introduction of bee hives in tents

A2 Individual Results

Table 10: Individual results of the evaluations of mortality (numbers of dead bees in the dead bee trap and on the linen sheet) in the test substance treatment

Date	ED	Mortality in the bee trap					Mortality on the linen					Mean mortality / tent and day
		Tent 1	Tent 2	Tent 3	Mean BT	STD BT	Tent 1	Tent 2	Tent 3	Mean Linen	STD Linen	
24APR01	1	10	1	2	4.3	4.9	0	5	1	2.0	2.6	6.3
25APR01	2	10	20	1	10.3	9.5	18	10	22	16.7	6.1	27.0
26APR01	3	3	15	0	6.0	7.9	5	0	5	3.3	2.9	9.3
27APR01	4	7	11	2	6.7	4.5	9	5	18	10.7	6.7	17.3
28APR01	5	5	5	0	3.3	2.9	9	1	8	6.0	4.4	9.3
29APR01	6	1	0	1	0.7	0.6	10	3	5	6.0	3.6	6.7
30APR01	7	2	3	2	2.3	0.6	17	4	11	10.7	6.5	13.0
Mean		5.4	7.9	1.1	4.8	5.5	9.7	4.0	10.0	7.9	6.4	12.7
STD		3.7	7.6	0.9	--	--	6.3	3.3	7.6	--	--	--
Total number of dead bees		38	55	8	--	--	68	28	70	--	--	--

ED = Evaluation day
BT = Bee traps
STD = Standard deviation

Table 11: Individual results of the evaluations of mortality (numbers of dead bees in the dead bee trap and on the linen sheet) in the control

Date	ED	Mortality in the bee trap					Mortality on the linen					Mean mortality / tent and day
		Tent 1	Tent 2	Tent 3	Mean BT	STD BT	Tent 1	Tent 2	Tent 3	Mean Linen	STD Linen	
24APR01	1	4	2	2	2.7	1.2	4	3	5	4.0	1.0	6.7
25APR01	2	16	7	15	12.7	4.9	24	32	6	20.7	13.3	33.3
26APR01	3	25	1	7	11.0	12.5	15	21	6	14.0	7.5	25.0
27APR01	4	13	6	10	9.7	3.5	13	19	11	14.3	4.2	24.0
28APR01	5	16	3	7	8.7	6.7	22	19	5	15.3	9.1	24.0
29APR01	6	1	3	3	2.3	1.2	6	10	2	6.0	4.0	8.3
30APR01	7	3	7	3	4.3	2.3	12	14	11	12.3	1.5	16.7
Mean		11.1	4.1	6.7	7.3	6.3	13.7	16.9	6.6	12.4	8.0	19.7
STD		8.8	2.5	4.6	--	--	7.5	9.2	3.3	--	--	--
Total number of dead bees		78	29	47	--	--	96	118	46	--	--	--

ED = Evaluation day
BT = Bee traps
STD = Standard deviation

Table 12: Average flight intensity (number of bees per tree/minute) in the three tents of the test substance treatment

Date	Time	ED	Tent 1	Tent 2	Tent 3	Mean (±STD)
24APR01	09:40 - 09:58	1	0.00*	0.00*	0.00*	3.22 ± 4.55
	12:40 - 12:52	1	0.00*	0.00*	1.33*	
	16:14 - 16:30	1	9.00	10.33	8.33	
Mean/day			3.00	3.44	3.22	--
25APR01	10:50 - 11:05	2	0.00	0.00	0.33	0.04 ± 0.11
	13:00 - 13:08	2	0.00	0.00	0.00	
	16:00 - 16:10	2	0.00	0.00	0.00	
Mean/day			0.00	0.00	0.11	--
26APR01	12:30 - 12:50	3	4.67	1.67	11.00	7.72 ± 4.30
	16:30 - 16:45	3	8.00	7.33	13.67	
Mean/day			6.33	4.50	12.33	--
27APR01	12:00 - 12:14	4	9.33	10.67	13.33	13.06 ± 5.20
	15:30 - 15:48	4	19.00	6.67	19.33	
Mean/day			14.17	8.67	16.33	--
28APR01	15:45 - 15:58	5	19.00	9.67	17.33	15.33 ± 4.98
29APR01	14:00 - 14:10	6	19.00	14.67	29.00	20.89 ± 7.35
30APR01	10:23 - 10:43	7	7.67	9.00	13.67	10.11 ± 3.15
Mean/tent/day (± STD)			10.05 ± 7.55			

ED = Evaluation day
STD/± = Standard deviation
* = Average number of forager bees on 25 flowers per tent. Because of a low number of forager bees on 25 flowers per tent the following assessments were carried out by counting the bees per tree.

Table 13: Average flight intensity (number of bees per tree/minute) in the three tents of the control

Date	Time	ED	Tent 1	Tent 2	Tent 3	Mean (±STD)
24APR01	10:08 - 10:22	1	0.00*	0.00*	0.67*	3.26 ± 4.66
	12:58 - 13:14	1	0.00*	0.00*	0.33*	
	15:55 - 16:07	1	9.33	10.33	8.67	
Mean/day			3.11	3.44	3.22	--
25APR01	11:10 - 11:20	2	0.00	0.00	0.00	0.00 ± 0.00
	13:12 - 13:25	2	0.00	0.00	0.00	
	16:15 - 16:25	2	0.00	0.00	0.00	
Mean/day			0.00	0.00	0.00	--
26APR01	12:55 - 13:10	3	6.33	3.67	3.67	6.61 ± 2.78
	16:40 - 16:58	3	10.33	6.33	9.33	
Mean/day			8.33	5.00	6.50	--
27APR01	12:20 - 12:35	4	9.67	14.33	11.33	10.95 ± 2.52
	15:55 - 16:10	4	7.00	10.67	12.67	
Mean/day			8.33	12.50	12.00	--
28APR01	16:07 - 16:28	5	9.67	7.33	9.33	8.78 ± 1.26
29APR01	14:20 - 14:35	6	19.67	21.00	20.00	20.22 ± 0.69
30APR01	10:57 - 11:12	7	11.67	15.67	17.33	14.89 ± 2.91
Mean/tent/day (± STD)			9.24 ± 6.58			

ED = Evaluation day
STD/± = Standard deviation
* = Average number of forager bees on 25 flowers per tent. Because of a low number of forager bees on 25 flowers per tent the following assessments were carried out by counting the bees per tree.

Table 14: Average flight intensity (entering and leaving the hives/minute) in the three tents of the test substance treatment

Date	Time	ED	Tent 1		Tent 2		Tent 3		Mean (±STD)
			Mean	STD	Mean	STD	Mean	STD	
24APR01	09:45 - 09:59	1	1.00	0.00	1.00	0.00	0.50	0.71	6.17 ±4.96
	12:41 - 12:54	1	7.50	2.12	8.00	4.24	9.00	1.41	
	16:15 - 16:32	1	14.00	1.41	10.00	5.66	4.50	2.12	
Mean/day			7.50		6.33		4.67		--
25APR01	10:50 - 11:05	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00
	13:00 - 13:08	2	0.00	0.00	0.00	0.00	0.00	0.00	
	16:00 - 16:10	2	0.00	0.00	0.00	0.00	0.00	0.00	
Mean/day			0.00		0.00		0.00		--
26APR01	12:33 - 12:52	3	9.00	7.07	12.50	10.61	8.50	6.36	9.92 ± 5.09
	16:35 - 16:45	3	11.50	0.71	6.50	2.12	11.50	4.95	
Mean/day			10.25		9.50		10.00		--
27APR01	12:04 - 12:18	4	12.00	5.66	11.50	4.95	8.00	2.83	15.58 ± 7.24
	15:35 - 15:45	4	20.50	7.78	22.50	7.78	19.00	7.07	
Mean/day			16.25		17.00		13.50		--
28APR01	15:48 - 15:57	5	17.50	7.78	19.50	7.78	19.00	9.90	18.67 ± 6.10
29APR01	14:00 - 14:15	6	10.50	4.95	15.00	1.41	15.50	4.95	13.67 ± 3.68
30APR01	10:26 - 10:50	7	10.00	4.24	7.50	0.71	7.00	2.83	8.17 ± 2.71
Mean/tent/day (± STD)			10.31 ± 7.58						

ED = Evaluation day
STD/± = Standard deviation

Table 15: Average flight intensity (entering and leaving the hives/minute) in the three tents of the control

Date	Time	ED	Tent 1		Tent 2		Tent 3		Mean (±STD)
			Mean	STD	Mean	STD	Mean	STD	
24APR01	10:10 - 10:26	1	1.50	2.12	1.00	1.41	1.50	0.71	8.72 ± 6.74
	13:02 - 13:15	1	10.50	0.71	5.50	0.71	11.50	2.12	
	15:58 - 16:05	1	15.00	2.83	14.00	1.41	18.00	8.49	
Mean/day			9.00		6.83		10.33		
25APR01	11:11 - 11:20	2	0.50	0.71	0.00	0.00	0.00	0.00	0.06 ± 0.24
	13:12 - 13:25	2	0.00	0.00	0.00	0.00	0.00	0.00	
	16:15 - 16:25	2	0.00	0.00	0.00	0.00	0.00	0.00	
Mean/day			0.17		0.00		0.00		
26APR01	12:58 - 13:12	3	8.00	1.41	4.50	0.71	7.50	4.95	9.50 ± 4.17
	16:52 - 16:58	3	10.00	2.83	12.50	4.95	14.50	0.71	
Mean/day			9.00		8.50		11.00		--
27APR01	12:25 - 12:37	4	10.00	1.41	16.00	8.49	15.50	2.12	14.83 ± 5.11
	16:00 - 16:15	4	20.50	3.54	16.00	4.24	11.00	6.36	
Mean/day			15.25		16.00		13.25		--
28APR01	16:05 - 16:14	5	26.00	5.66	7.00	1.41	12.50	0.71	15.17 ± 8.33
29APR01	14:20 - 14:35	6	8.00	1.41	9.00	2.83	18.00	5.66	11.67 ± 5.22
30APR01	10:55 - 11:15	7	12.50	14.85	12.00	1.41	15.50	9.19	13.33 ± 6.69
Mean/tent/day (± STD)			10.47 ± 7.55						

ED = Evaluation day
STD/± = Standard deviation

Table 16: Brood development of the test substance colonies

	Colony Tent 1	Colony Tent 2	Colony Tent 3
Prior to introducing bees in tents: 23APR01			
Strength (No. of combs covered with bees)	3.5	3.5	3.5
No. Of combs covered with brood	3	3	3
Average amount of egg stage in %	28.33	21.67	16.67
Average amount of larval stage in %	18.33	8.33	5.00
Average amount of capped stage in %	16.67	20.00	23.33
After introducing bees in tents: 30APR01			
Strength (No. of combs covered with bees)	3.5	3.5 – 4.0	3.5 – 4.0
No. Of combs covered with brood	3	4	2
Average amount of egg stage in %	16.67	10.00	12.50
Average amount of larval stage in %	20.00	11.25	25.00
Average amount of capped stage in %	23.33	12.50	27.50

Table 17: Brood development of the control colonies

	Colony Tent 1	Colony Tent 2	Colony Tent 3
Prior to introducing bees in tents: 23APR01			
Strength (No. of combs covered with bees)	3.5	3.0	3.5
No. of combs covered with brood	4	3	5
Average amount of egg stage in %	10.00	11.67	13.00
Average amount of larval stage in %	6.25	10.00	10.00
Average amount of capped stage in %	13.75	20.00	11.00
After introducing bees in tents: 30APR01			
Strength (No. of combs covered with bees)	3.5	3.0	3.5
No. of combs covered with brood	4	3	4
Average amount of egg stage in %	2.50	21.67	18.75
Average amount of larval stage in %	20.00	15.00	10.00
Average amount of capped stage in %	10.00	16.67	8.75

Table 18: Results of recording of the flowering stages

Date	ED	BBCH code of the apple flowers
24APR01	1	61 – 62
25APR01	2	61 – 62
26APR01	3	63 – 64
27APR01	4	64 – 65
28APR01	5	65
29APR01	6	65
30APR01	7	65

ED: Evaluation day after the beginning of bee exposure

*Remark:

BBCH codes	Explanation
61	10 % of flowers open
62	20 % of flowers open
63	30 % of flowers open
64	40 % of flowers open
65	Full flowering 50 % of flowers open, older petals falling

A3 Pictures

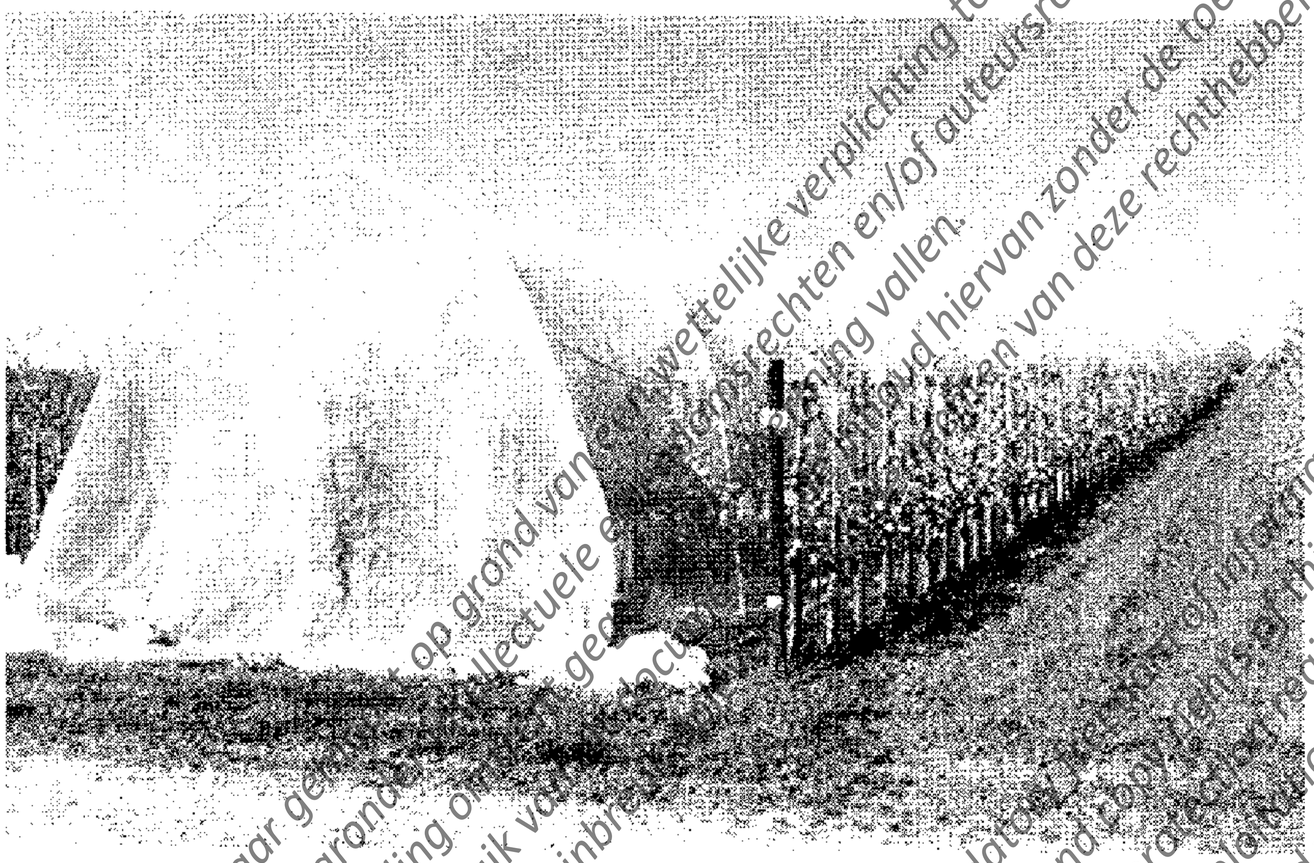


Figure 9: Arrangement of the tunnels

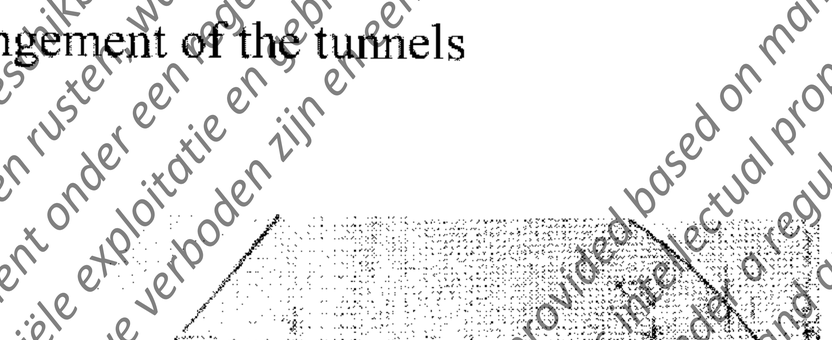


Figure 10: Apple tree espalier in one tunnel of the test substance treatment

A4 Certificates

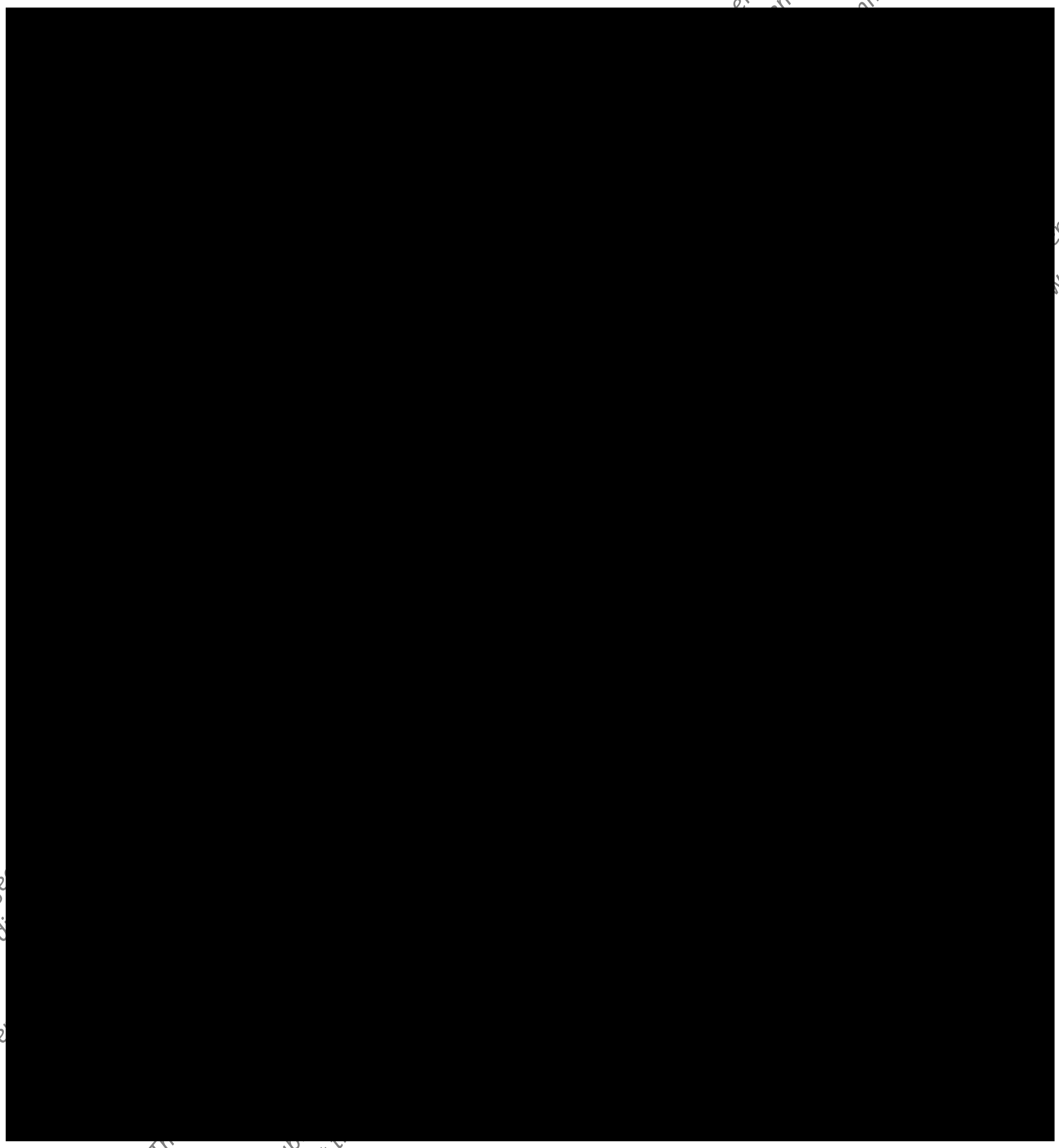


Figure 11: Certificate of analysis of Confidor SL 200

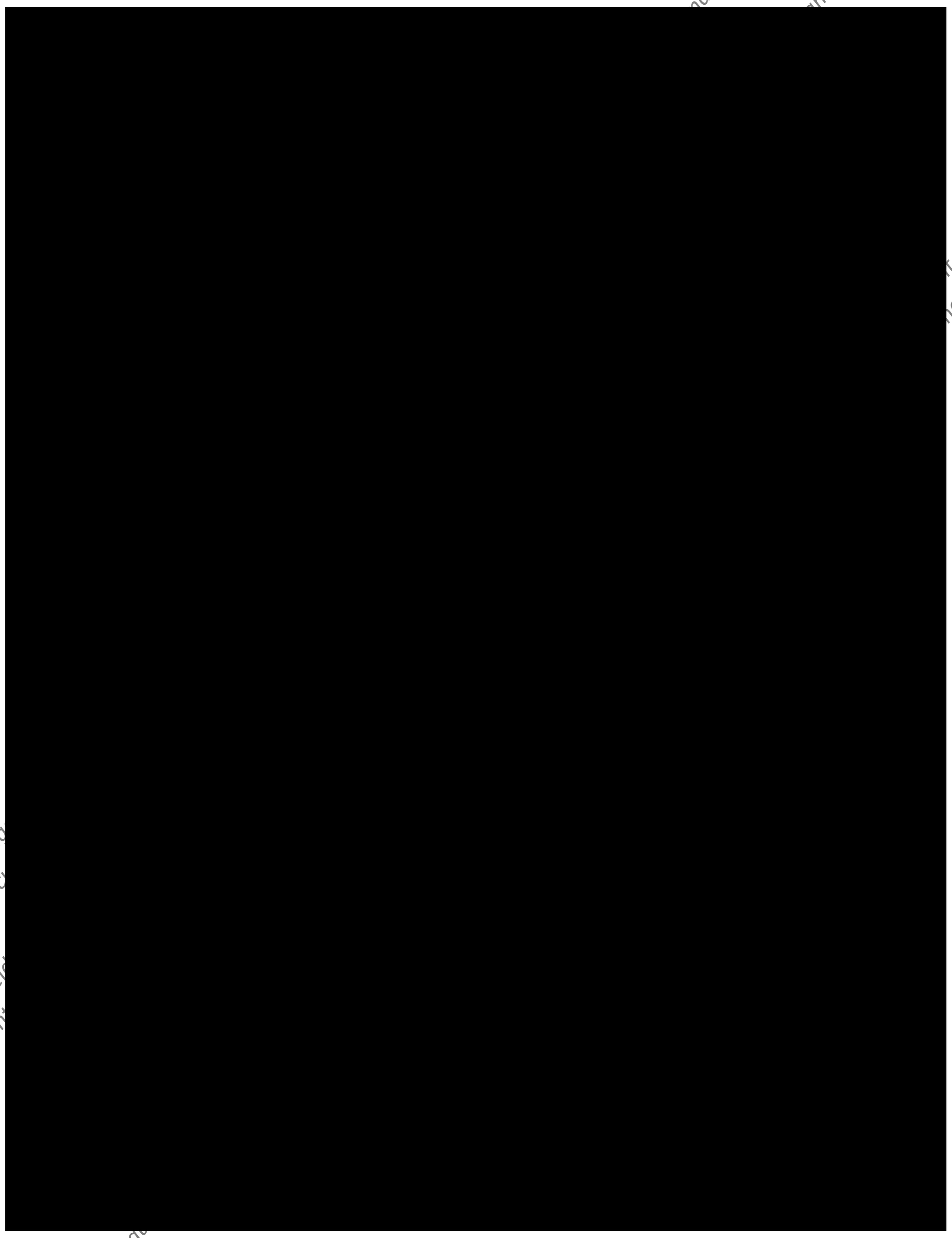


Figure 12: GLP Certificate of testing facility