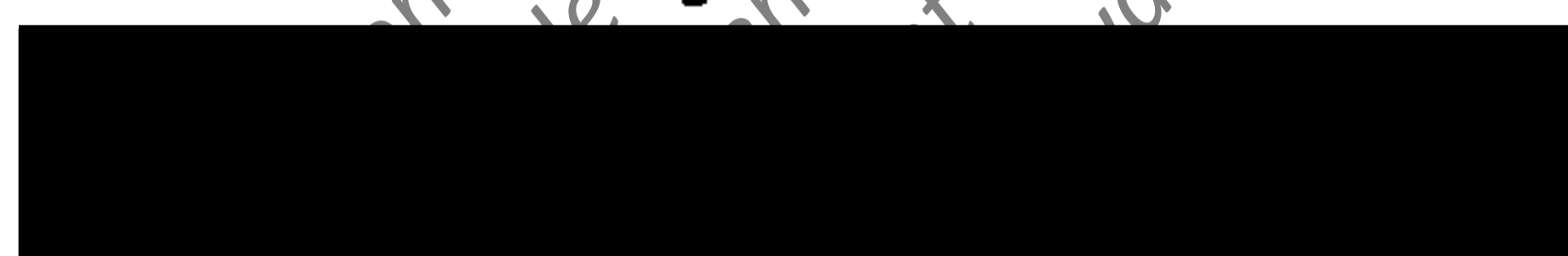


**Final Report****Assessment of Side Effects of Confidor SL 200
on the Honey Bee (*Apis mellifera* L.) in Apple Orchard Following
Application before flowering (Mouse-Ear Stage) of the Crop****Data Requirements**

Based on BBA Guideline VI, 23.1 and EPPO Guideline No. 170

Study Director**Date**

05DEC2001

Testing Facility

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Study Identification Code

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

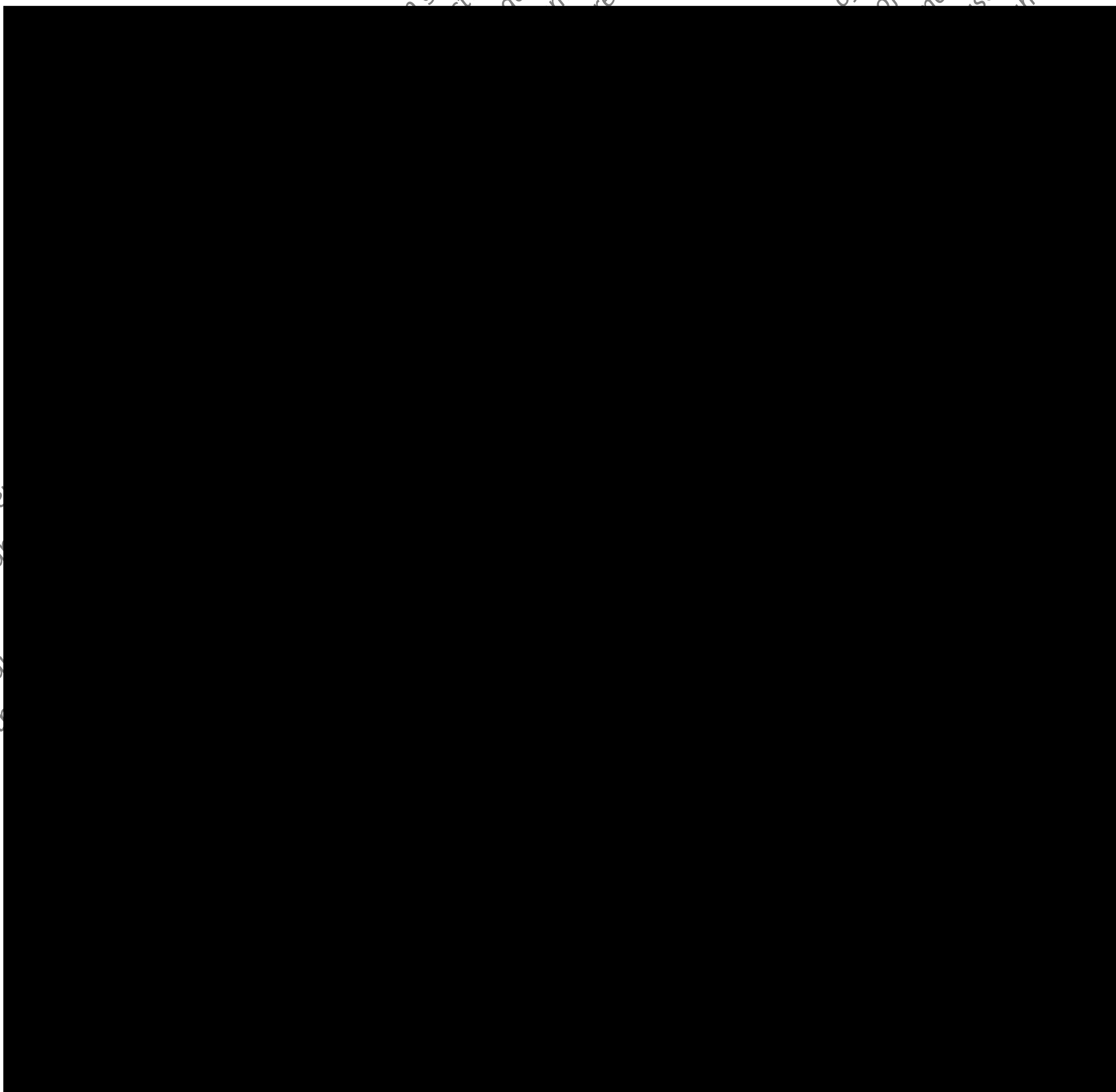




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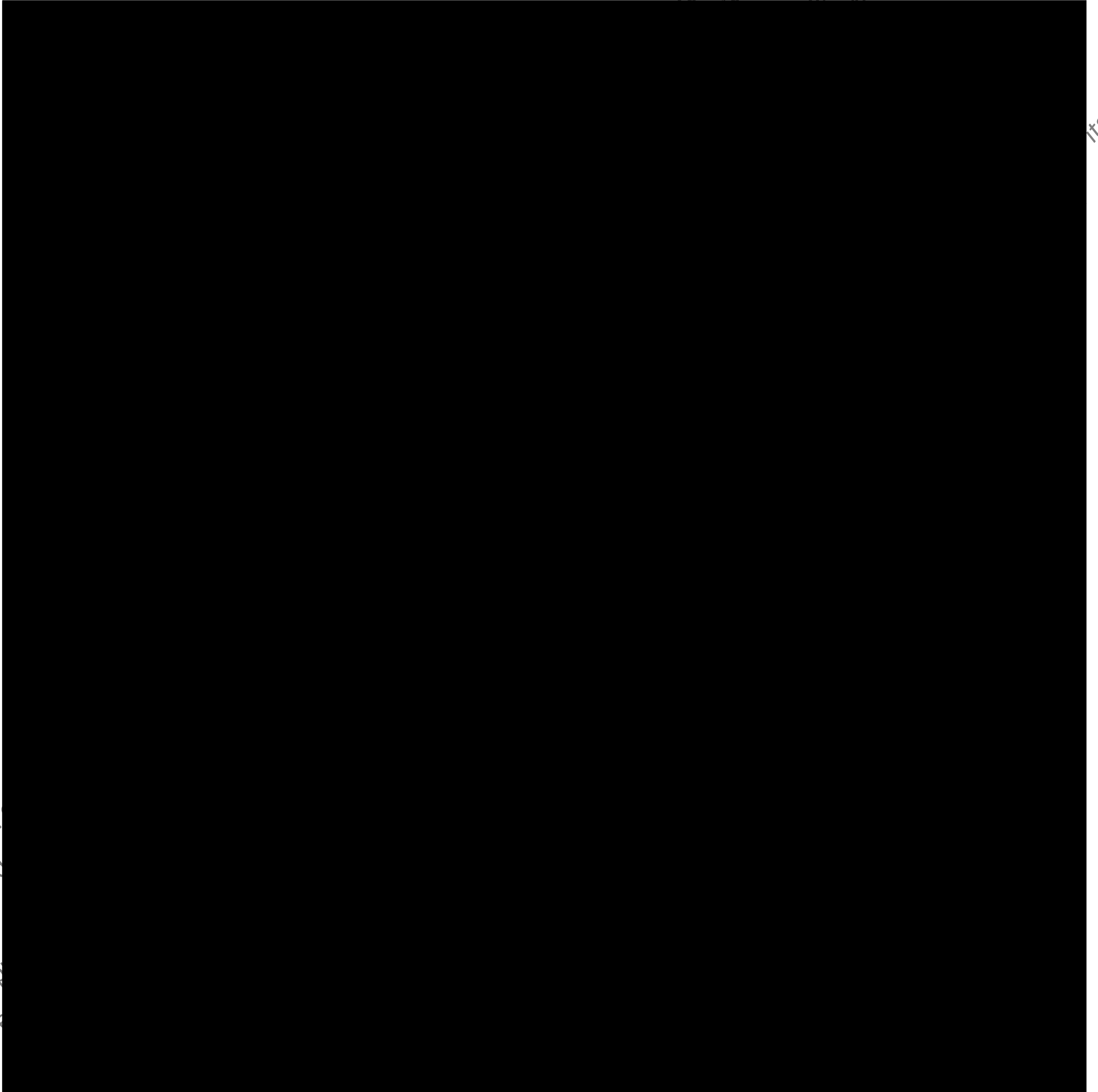
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1 Summary

Report: [REDACTED]: 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unpublished report No: 20011099/01-BFEU,
05/12/2001

Guidelines: BBA Guideline VI, 23-1 and 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 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 (the amount of water was adapted to the height and the growth stage of the trees according to Good Agricultural Practice). The application was performed at the mouse-ear stage of the apple trees (BBCH-code 10) on 30MAR2001. An untreated orchard of apple trees from the same variety served as control. At the start of full flowering (29APR2001) four colonies were placed between the rows of each test field.

Mortality, foraging activity, behaviour, and condition of the colonies, the development of the bee brood and the weight changes of the colonies 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 field to those in the control field regarding the following observations:

- Mortality 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
- Weight changes of the colonies

Dates of work: 30MAR2001 – 12JUN2001

Findings

Effect on honey bee mortality:

In the Confidor SL 200 treated group as well as in the control group the mean mortality increased from ED 2 until the end of the observation period (ED 7). The mean mortality rose up to a mean maximum of 25.3 dead bees per colony/day in the Confidor SL 200 treated group compared to a mean maximum of 43.5 dead bees/colony/day in the control group, both observed on ED 7. On every assessment day the mean values of mortality observed in the test substance treatment group were lower than in the control group.

Effects on honey bee flight intensity:

During the entire exposure period the mean flight intensity in the test substance treated group was similar or on a higher level compared to the control. By comparing the overall mean of flight intensity a value of 5.0 bees/tree/minute was found in the test substance treated group compared to 3.6 bees visiting the flowers in the control group.

The mean flight intensity observed in front of the hives increased during the first three assessment days (ED 1 to 3) in both treatment groups and remained on a high level from ED 3 to 5. On day 6 and 7 after start of exposure the mean flight intensity observed at the front of the hives was on a lower level compared to the previous days. Only a slight difference between the test substance treated group and the control group occurred concerning the mean flight intensity in front of the hives over the entire test period (33.62 bees leaving/entering the hive per minute in the treated group and 37.32 bees leaving/entering the hive per minute in the control group).

Effects on honey bee brood development:

In the bee brood development no abnormal difference which could be attributed to the influence of the test substance were observed between the test substance and control treatment.

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.

Weight of the colonies

No remarkable observations were made regarding the weight changes of the Confidor SL 200 treated hives compared to the control hives.

Conclusion

The treatment of apple trees at the mouse-ear stage with Confidor SL 200 at the 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 field study.

2 Time Schedule

Study initiation date:	29MAR2001
Start of the experimental phase:	30MAR2001
End of the experimental phase:	12JUN2001
Draft report:	16NOV2001
Study completion date:	05DEC2001

3 Study Objective

The following study was designed to determine the effects of Confidor SL 200 on the honey bee (*Apis mellifera* L.) under 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 (amount of water was adapted to the height of the trees). The application was performed at the mouse-ear stage of the apple trees (BBCH-code 10). The exposure of the bees begun at start of full flowering of the trees (BBCH-code 64 - 65).

This GLP compliant study was conducted based on the guideline for the testing of plant protection products for registration of the Federal Biological Research Centre for Agriculture and Forestry, Federal Republic of Germany (BBA), part VI, 23-1 (STUTE *et al.* 1991) and the guideline of the European and Mediterranean Plant Protection Organisation No. 170 (EPPO, 1992).

4 Material and Methods

4.1 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 and is therefore not reported.

4.2 Control

The control plot was untreated.

4.3 Test Organism

As test organism the honey bee, *Apis mellifera* (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.

4.4 Principles of the Study

The test substance Confidor SL 200 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). The amount of water was adapted to the height and the growth stage of the trees according to Good Agricultural Practice. An untreated orchard with apple trees served as control.

Bee colonies were placed between the rows of the apple trees in the test fields at start of full flowering (BBCH-code 64 - 65) of the trees.

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

In the trial the effect of the test substance was examined on bee colonies used for honey production.

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

The influence of the test substance was evaluated by comparing the bees of the test substance treatment to the bees of the control treatment.

4.5 Description of the Test Method

4.5.1 Test Location and Crop

The trial was conducted in Germany near Karlsruhe at the location Augustenberg (see Figure 1 and Table 1).



Figure 1: Location of the test site in Germany

Table 1: Description of the test site

Location	Augustenberg
Zip code	76227
Region	Baden-Württemberg
Country	Germany
Meters above sea level	150
Slope	0 - 2 %

The size of the test substance treated orchard was 2600.5 m² with approx. 925 apple trees. The size of the untreated control field was 3400 m² with approx. 940 apple trees. The distance between the test field and the control field was about 600 m. Both test fields were surrounded by different varieties of apple trees which were in flowering during the test period. Details about the crop used see in Table 2.

Table 2: Description of the crop at the test site

Crop	Apple
Variety	Braeburn, Golden Delicious
Date of planting	Spring 1995; Spring 1997
Distance between rows	3.5 m
Distance between trees in the row	1.0 m
Height of trees at start of bee testing	approx. 2.5 m

4.5.2 Design and Lay-out of the Test

The study was carried out with the treatments given in Table 3.

Table 3: Different test treatments

Treatment	Amount per ha	Application rate of water per ha	No. of colonies
Confidor SL 200	0.105 kg a.s.	500 L*	4
Control	--	--	4

*the amount of water was adapted to the height and the growth stage of the trees according to Good Agricultural Practice

4.5.3 Experimental Bee Colonies

Four normally developed, queen-right bee colonies were used per treatment. Each colony contained one brood and one honey body with in total 20 frames (5 – 8 brood frames). The colonies were provided from a beekeeper in Germany: Berthold Nengel, Dahlheim, Rheinland-Pfalz.

In front of the hives bee traps were fixed to record the number of dead bees.

The bee colonies were placed in each apple orchard at start of full flowering of the crop (29APR01).

4.5.4 Application of the Test Substance

The application of the spray suspension was carried out with a spraying equipment used for commercial applications (see Table 4).

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 recorded in the raw data.

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.

The environmental conditions (e.g. temperature, relative humidity and wind speed) were recorded during application.

Table 4: Details of treatment

		Test Substance
Date		30MAR2001
Time	[h:min]	9:40 – 9:52
Device		
Sprayer		Sprayer mounted on a tractor
Trade name of sprayer		Meyer
Nozzles (No./type)		10 x AlbuZ ATR red
Pressure	[bar]	9.5
Application speed	[km/h]	6.0
Technical faults		no
Dosage		
Plot size	[m ²]	2600.5
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]	547.97
Environment/Crop		
Temperature	[°C]	8.7 – 10.6
Humidity	[%]	64 - 72
Wind speed	[m/s]	1.0
Rainfall that day/next day	[mm]	0.9*/0.0
Clouding	[%]	90
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

4.5.5 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)

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).

4.6 Mode of Assessment

4.6.1 Mortality in Front of the Bee Hives and in the Field

In order to record the number of dead honey bees in the test and control colonies water-permeable linen sheets of 1.5 m width and about 3 m length were spread out in front of the hives. 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 were carried out of the hives.

The observations of mortality were carried out according to the scheme given in Table 5.

Table 5: Evaluation of mortality

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

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

4.6.2 Flight Intensity of the Bees in the Field and in Front of the Hives

The observations of the flight intensity in the field, which started one day after the bee hives were set-up, took place at five marked trees distributed over the test and control field. At each assessment time the number of bees that were both foraging on the marked trees and flying over the trees 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 6. The assessments were performed approx. at the same time in both fields.

Table 6: Evaluation of flight intensity

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

4.6.3 Conditions of the Colonies Development of the Bee Brood

The condition of the colonies and the development of the bee brood was checked 2 days before the set-up of the test colonies and 8 days as well as 4 weeks after the set-up of the colonies in the orchards. After the period of assessments the hives were placed to a location where no pesticides were used in flowering crops.

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.

The amount of eggs, larvae and capped brood was given in percent of total brood population for each type of brood.

4.6.4 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 before as well as after application once a day.

Bees with pollen loads were counted and identified in front of two colonies. The assessment was performed by counting the total number of bees returning to the hive with pollen loads and in parallel the bees entering the hive with apple pollen loads. The assessment was carried out two times (ED 2 and ED 4) during the test and for one minute per hive and assessment date (see Table 17 and Table 18 in the Appendix A2).

4.6.5 Flowering Stage of the Crop and Fruit Buds

The stages of blooming of the apple trees (BBCH-code) was recorded every second day and were documented in the raw data (see Table 19 in the Appendix).

Additionally the fruit buds in the different test fields were assessed three times on 5 marked trees after the set-up of the test colonies, 6 days after the set-up of the test colonies and 15 days as well as 6 weeks after the set-up of the colonies in the orchards (see Table 20 and Table 21 in the Appendix). The assessments were carried out at the same areas as for the evaluation of flight intensity in the field.

4.6.6 Weight of the Test Colonies

The weight changes (increase or decrease) of three colonies per treatment were recorded continuously by means of a beam scale during the entire test period (see Table 22 and Table 23 in the Appendix).

4.7 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
- Development of the bee brood
- Flight intensity in the orchard and in front of the hives
- Behaviour of the bees at the entrance of the hives
- Weight changes of the colonies

Data on mortality will usually be evaluated one sided higher to analyse for differences between the treatment group and control group.

Since the mortality data (dead bee trap and linen sheets) in the test substance treatment was on a lower level on all assessment days compared to the control treatment no statistical evaluation of the results was carried out.

5 Deviations from the Study Plan

The study was performed according to the study plan dated 29MARCH01 and with the following deviations:

1. Observations at the Entrance of the Hives (item 2.6.4 of the study plan)

Deviation: The assessment of bees with (apple) pollen loads returning to the hive was performed only two times on ED 2 and 4.

Reason: On ED 6 the weather conditions were cool and windy and no bees with pollen loads could be observed.

Effect on the study: Data of only two assessment dates are available.

This report reflects the conduct of this study.

6 Dates of the Test

The important dates of the trial are given in Table 7.

Table 7: Dates of the field trial

Activity	ED	Date
Application of the test substance	-30	30MAR2001
1 st brood assessment	-2	27APR2001
Set-up of test hives	0	29APR2001
1 st evaluation of mortality and flight intensity	1	30APR2001
1 st assessment of fruit buds	6	05MAY2001
Last evaluation of mortality and flight intensity	7	06MAY2001
2 nd brood assessment	8	07MAY2001
2 nd assessment of fruit buds	15	14MAY2001
Last brood assessment	29	28MAY2001
Last assessment of fruit buds	44	12JUN2001

Remark: ED = Evaluation day

7 Results

7.1 Mortality

Figure 2 shows the average mortality (number of dead bees/colony/day) in the test substance and in the control treatment (see also Table 9 and Table 10 in the Appendix A2). The evaluation period of 7 days is identified by ED (evaluation day) 1 to 7.

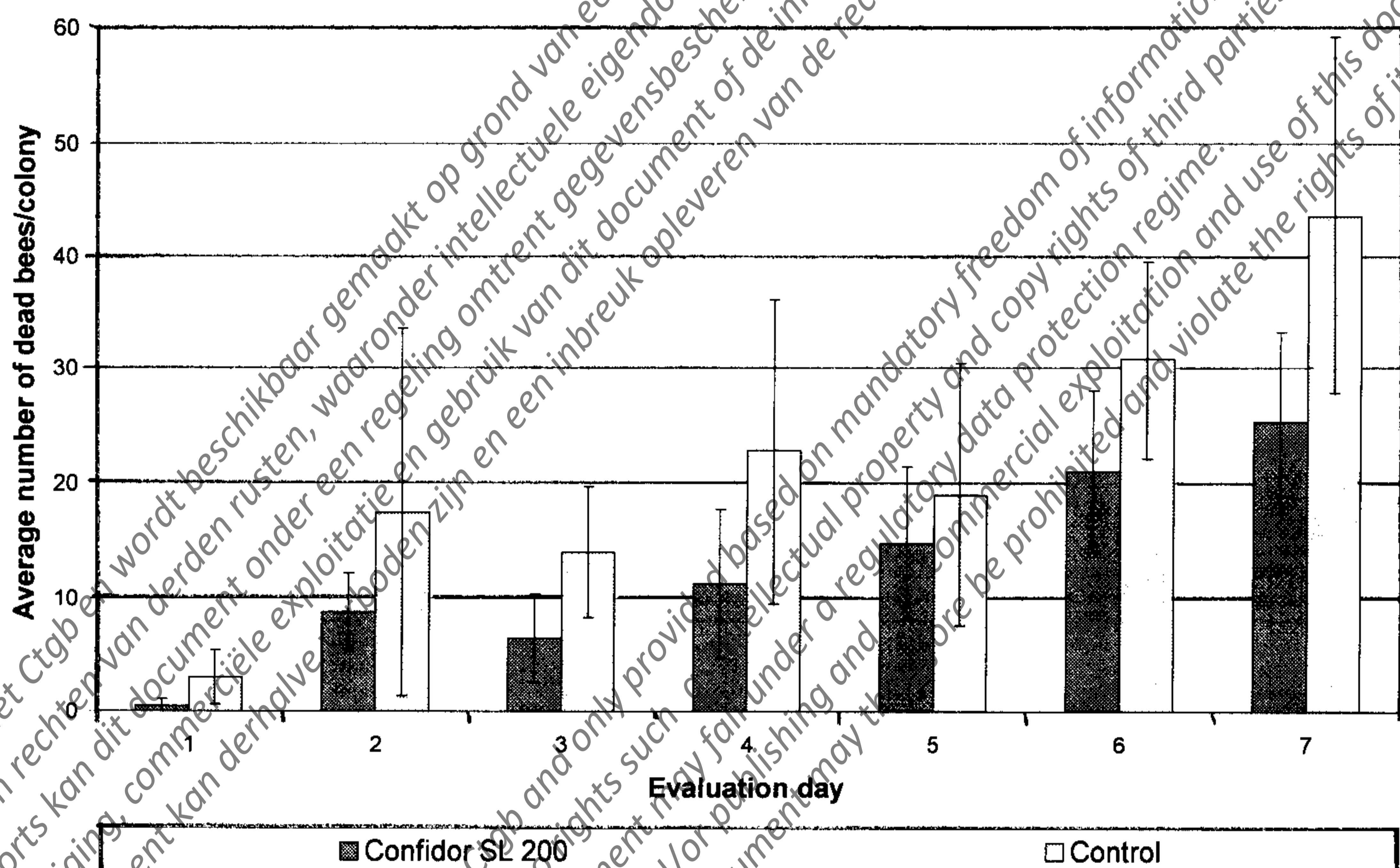


Figure 2: Average number of dead bees (\pm STD) collected in the dead bee traps and on the water permeable linen sheet in front of the bee hives in the test substance and control treatment from evaluation day 1 to 7 after the start of bee exposure

The mean mortality per day was 0.5 and 8.8 dead bees/colony in the test substance treatment group and 3.0 and 17.5 dead bees/colony in the control group on ED 1 and 2, respectively. In the Confidor SL 200 treated group as well as in the control group the mean mortality increased from ED 2 until the end of the observation period (ED 7). The mean mortality rose up to a mean maximum of 25.3 dead bees per colony/day in the Confidor SL 200 treated group compared to a mean maximum of 43.5 dead bees/colony/day in the control group, both observed on ED 7. The mean values of mortality observed in the test

substance treatment group were lower than in the control group on every assessment day.

The average daily mortality during the entire observation days (ED 1 to ED 7) was 12.6 dead bees/colony in the Confidor SL 200 treatment group and 21.5 dead bees/colony in the control group (see Table 9 and Table 10 in the Appendix).

7.2 Flight Intensity

In the crop

Figure 3 shows the average flight intensity (average number of forager bees/tree/minute) in the test substance treatment and in the control (see also Table 11-Table 12 in the Appendix A2). The evaluation period of 7 days is identified by ED 1 to 7 (evaluation days).

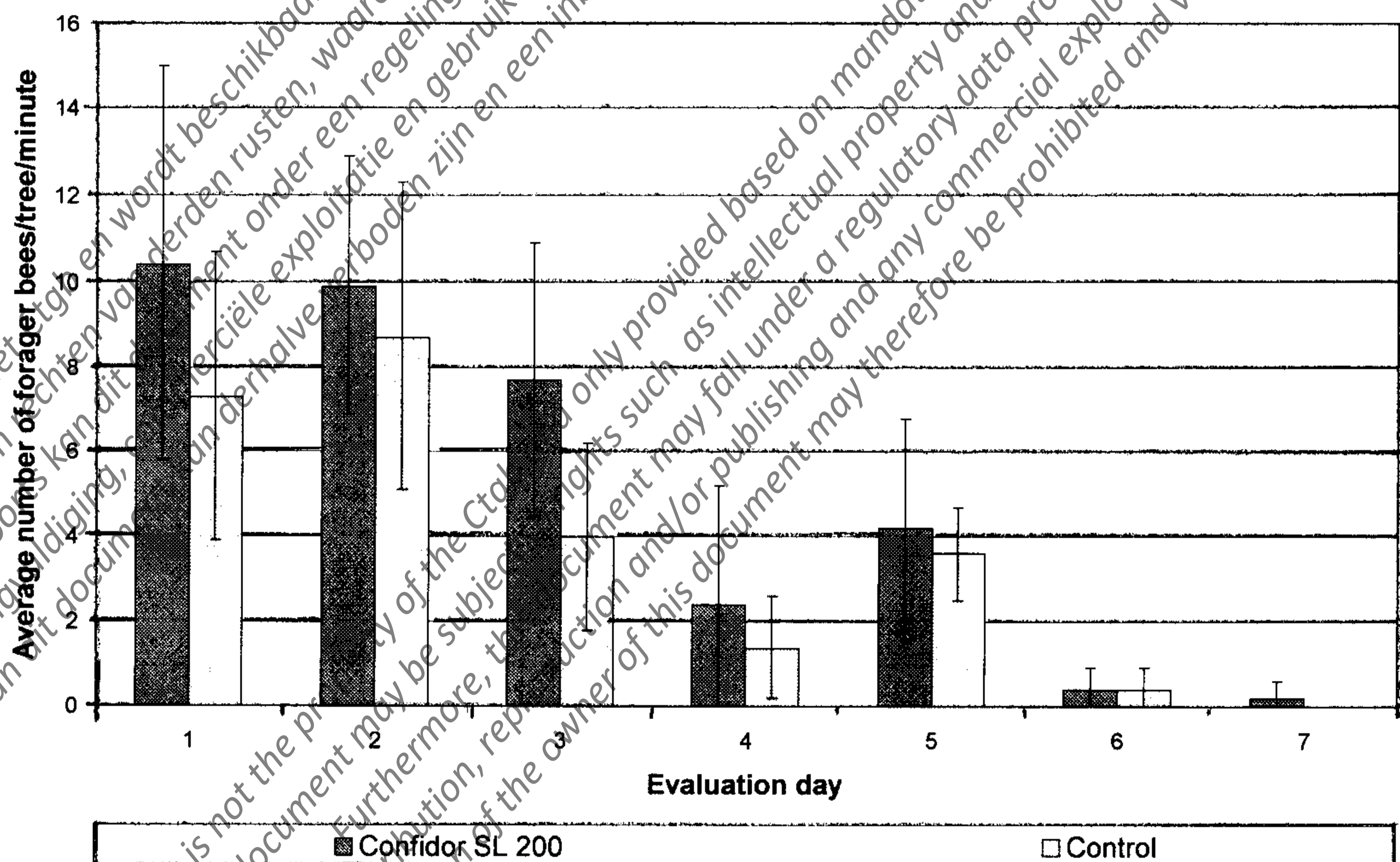


Figure 3: Average flight intensity (number of forager bees/tree/minute) (\pm STD) in the test substance and control treatment from evaluation day 1 to 7 after the start of bee exposure

During the entire exposure period the mean flight intensity in the test substance treated group was similar or on a higher level compared to the control. On ED 1 and 2 the mean flight intensity was 9.9 to 10.4

bees/tree/minute in the Confidor SL 200 treatment group and 7.3 to 8.7 bees/tree/minute in the control group. A decrease could be observed on the days ED 3 to 7. On ED 3 to 5, 7.7 - 4.2 bees/tree/minute were observed in the test substance treatment and 4.0 - 3.6 bees/tree/minute were counted in the control group. Almost no bees were counted per tree and minute on the last two evaluation days ED 6 and 7 which can be attributed to the high cloud cover and the low temperatures on these days.

By comparing the overall mean of flight intensity a value of 5.0 bees/tree/minute was found in the test substance treated group which was higher than that of the control group (3.6 bees/tree/minute).

In front of the hive

Figure 4 shows the average flight intensity in front of the hives (number of bees entering/leaving the hive/minute) in the test substance treatment and in the control (see also Table 13 to Table 14 in the Appendix). The evaluation period of 7 days is identified by ED 1 to 7 (evaluation days).

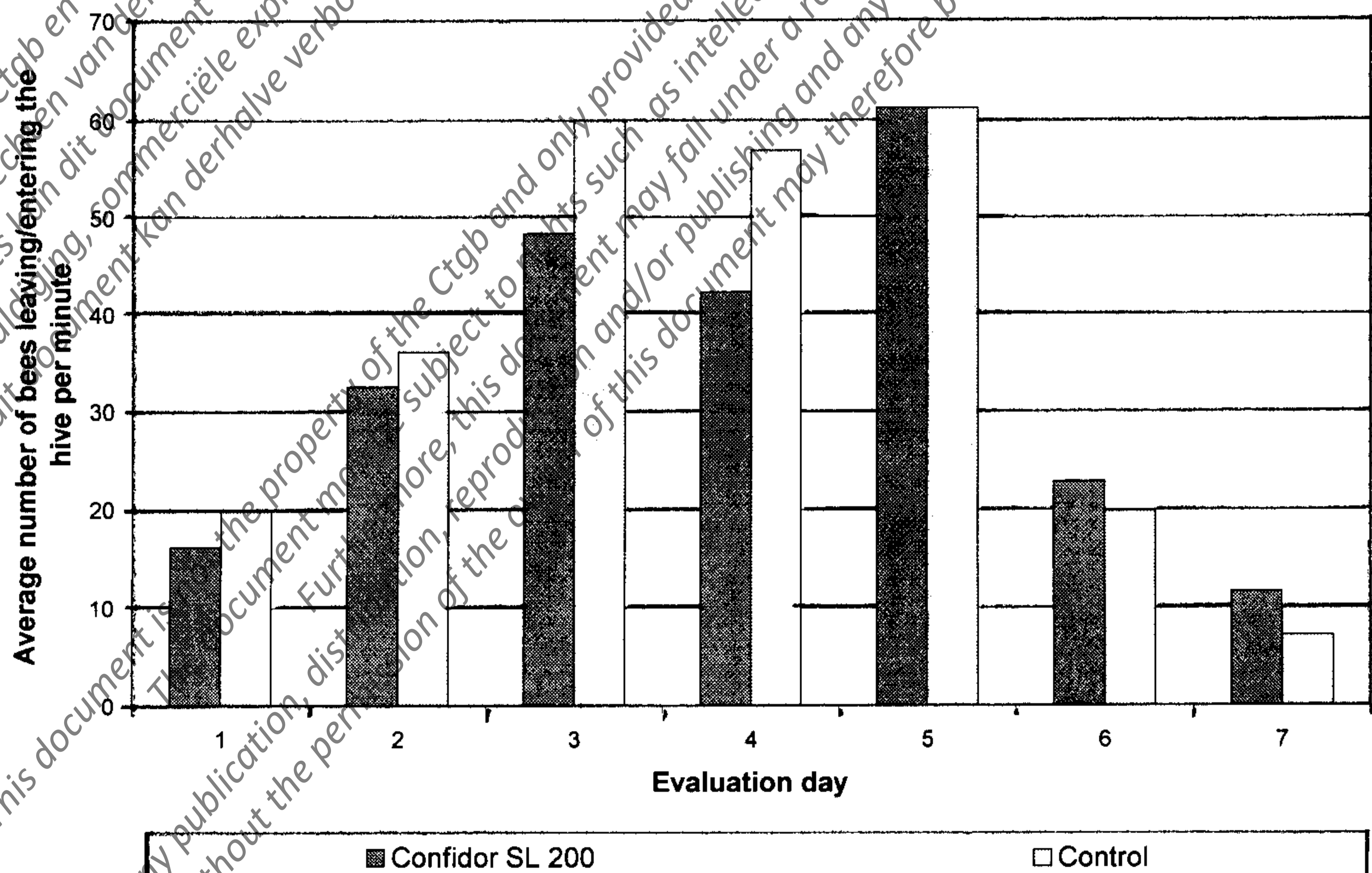


Figure 4: Average flight intensity (number of bees leaving/entering the hive/minute) in the test substance and control treatment from evaluation day 1 to 7 after the start of bee exposure

The mean flight intensity observed in front of the hives increased during the first three assessment days (ED 1 to 3) in both treatment groups and remained on a high level from ED 3 to 5 (42.31 to 61.25 bees leaving/entering the hive per minute in the treated group and 56.94 to 61.13 bees leaving/entering the hive per minute in the control). On day 6 and 7 after start of exposure the mean flight intensity observed at the front of the hives was on a lower level compared to the previous days. In the Confidor SL 200 treatment group an average of 22.88 to 11.75 bees leaving/entering the hive per minute were counted and 19.88 to 7.25 bees leaving/entering the hive per minute were calculated as mean of the control hives.

Only a slight difference occurred concerning the mean flight intensity in front of the hives over the entire test period (33.62 bees leaving/entering the hive per minute in the treated group and 37.32 bees leaving/entering the hive per minute in the control group).

7.3 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 every colony of the test substance group and control group (see Table 15 - Table 16 in the Appendix A2). The strength of the colonies was on a constant level over the observation period and the number of combs covered with bees ranged between 7 and 9 in the colonies of both treatment groups. The number of combs covered with brood increased in the hives of the Confidor SL 200 treatment group and in hive No. 2 and 4 of the control group. At the last assessment no eggs and larval stages could be assessed in hive No. 1 and No. 3 of the control group which is the evidence that the queens of these hives died after the second brood assessment.

The continued presence of eggs in all other colonies showed that the queens were in good condition. No differences in the condition of the colonies or the brood development between the colonies of the test substance group and hive No. 2 and No. 4 of the control group were noticed.

7.4 Behaviour of the Bees

The bees visiting the treated apple flowers showed normal and intensive pollen and nectar collection with no differences to the control. On all evaluation days no abnormal behaviour of the bees in the

Confidor SL 200 treated orchard and around the colonies was observed compared to the control treatment.

7.5 Pollen Collection of the Bees

The results of the observation of bees returning to the hive with pollen loads are summarised in Table 17 and Table 18 in Appendix.

The number of bees with pollen loads entering the hive per minute ranged between 11 and 19 in the test substance group and between 8 and 20 in the control group on the two assessment dates. In the Confidor SL 200 treatment group all pollen which was collected by the bees derived from the apple trees at the first assessment on ED 2. At the second assessment on ED 4 the bees of hive No. 6 collected only 11.1 % apple pollen, but the percentage of apple pollen of the other two hives observed ranged between 78.9 and 100.

In the control treatment the main part of pollen collected derived from the apple trees at all assessment days and times (87.5 to 100.0 %).

The high portions of apple pollen collected indicated that the bees visited the apple plants and that an exposure to possible residues of Confidor SL 200 was guaranteed in the test substance treated group.

7.6 Fruit buds

The results of the evaluation of the fruit buds in the test substance and in the control field are summarised in Table 20 and Table 21 in the Appendix. From the first evaluation to the second evaluation a similar development concerning the relative number of fruit buds could be observed in the test substance and in the control field. The high amount of fruit buds in both treatments is the result of a good pollination activity of the bees and other insects in the field, which is comparable in the Confidor SL 200 treatment group and in the control group.

7.7 Weight of the Test Colonies

The continuously recorded weights per day of the colonies No. 5, 6 and 7 of the test substance treated group and of the colonies No. 1, 2 and 3 in the control group are presented in Table 22 and Table 23 in the Appendix. The weight of these colonies was measured during the exposure period from ED 0 to ED 7. The weight gain or loss of all six hives observed was comparable. In the Confidor SL 200 treatment

group a relative weight gain/loss between the first and the last weighing of +1.5 %, -1.6 % and -2.0 % occurred. The relative weight difference observed in the control group was -4.5 %, +1.6 % and -4.1 %.

No remarkable observations were made regarding the weight changes of the Confidor SL 200 treated hives compared to the control hives.

8 Discussion and Conclusions

Mortality

In the Confidor SL 200 treated group as well as in the control group the mean mortality increased from ED 2 until the end of the observation period (ED 7). The mean mortality rose up to a maximum of 25.3 dead bees per colony/day in the Confidor SL 200 treated group compared to a maximum of 43.5 dead bees/colony/day in the control group, both observed on ED 7. On every assessment day the mean values of mortality observed in the test substance treatment group were lower than in the control group.

Flight intensity

During the entire exposure period the mean flight intensity in the test substance treated group was similar or on a higher level compared to the control. By comparing the overall mean of flight intensity a value of 5.0 bees/tree/minute was found in the test substance treated group compared to 3.6 bees visiting the flowers in the control group.

The mean flight intensity observed in front of the hives increased during the first three assessment days (ED 1 to 3) in both treatment groups and remained on a high level from ED 3 to 5. On day 6 and 7 after start of exposure the mean flight intensity observed at the front of the hives was on a lower level compared to the previous days. Only a slight difference between the test substance treatment and the control group occurred concerning the mean flight intensity in front of the hives over the entire test period (33.62 bees leaving/entering the hive per minute in the treated group and 37.32 bees leaving/entering the hive per minute in the control group).

Brood development

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 every colony of the test substance group and control. The strength of the colonies was on a constant level over the observation in both treatment groups. The number of combs covered

with brood increased in the hives of the Confidor SL 200 treatment group and in hive No. 2 and 4 of the control group. At the last assessment no egg of larval stages could be assessed in hive No. 1 and No. 3 of the control group which is the evidence that the queens of these hives died after the second brood assessment.

The continued presence of eggs in all other colonies showed that the queens were in good condition. No differences in the condition of the colonies or the brood development between the colonies of the test substance group and hive No. 2 and No. 4 of the control group were noticed.

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.

Pollen Collection of the Bees

The high portions of apple pollen collected indicated that the bees visited the apple plants and that an exposure to possible residues of Confidor SL 200 was guaranteed in the test substance treated group.

Fruit buds

The high amount of fruit buds in both treatments is the result of a good pollination activity of the bees, which is comparable in the Confidor SL 200 treatment group and in the control group.

Weight of the colonies

No remarkable observations were made regarding the weight changes of the Confidor SL 200 treated hives compared to the control hives.

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.

STUTE, K., BOCK, K.-D., BRASSE, D., DRESCHER, W., DUSTMANN, J.-H., MAUTZ, D., PINSDORF, W., SCHULZ, A., VORWOHL, G. (1991): Auswirkungen von Pflanzenschutzmitteln auf die Honigbiene.- Biologische Bundesanstalt für Land- und Forstwirtschaft Bundesrepublik Deutschland, Richtlinien für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren, Teil VI, 23-1.

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 (1 x)

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12 Appendix

A1 General Information

Table 8: Weather conditions during the trial

(to be continued)

Date	ED	Ø Temperature* min/max [°C]	Precipitation* [mm]	Cloud formation at time of evaluation [%]
30MAR2001	-	5.8 / 11.1	0.9	90
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	0.1	-
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	-	4.4 / 19.3	0.1	-
25APR2001	-	9.4 / 12.9	7.8	-
26APR2001	-	7.6 / 14.5	0.7	-
27APR2001	-	6.3 / 17.4	1.0	-

ED = Evaluation days

*temperature and precipitation was recorded 9 km from the test site Augustenberg by a weather station in Karlsruhe (Meteo-Media-Messnetz)

**Table 8: Weather conditions during the trial
(continued)**

Date	ED	Ø Temperature* min/max [°C]	Precipitation* [mm]	Cloud formation at time of evaluation [%]
28APR2001	-	10.4 / 14.4	5.6	-
29APR2001	-	9.6 / 19.7	1.3	-
30APR2001	1	10.4 / 22.6	0.0	10-30
01MAY2001	2	9.9 / 22.2	0.0	0-30
02MAY2001	3	9.8 / 27.4	0.0	5
03MAY2001	4	13.0 / 21.1	0.0	20-80
04MAY2001	5	12.5 / 22.0	0.0	70
05MAY2001	6	11.2 / 13.2	0.0	100
06MAY2001	7	7.9 / 9.9	0.3	100

ED = Evaluation days

*temperature and precipitation was recorded 9 km from the test site Augustenberg by a weather station in Karlsruhe (Meteo-Media-Messnetz)

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A2 Individual Results

Table 9: Individual results of the evaluations of mortality (numbers of dead bees in the dead bee traps and linen sheets in front of the hives) in the test substance treatment

Date	ED	Mortality (numbers of dead bees)					Mean number of dead bees/ colony and day (\pm STD)
		Linen sheets in front of the hives	Hive 5	Hive 6	Hive 7	Hive 8	
		LS	BT	BT	BT	BT	
30APR01	1	0	0	1	1	0	0.5 \pm 0.6
01MAY01	2	7	3	11	8	6	8.8 \pm 3.4
02MAY01	3	1	10	4	9	2	6.5 \pm 3.9
03MAY01	4	3	6	19	5	12	11.3 \pm 6.5
04MAY01	5	6	6	16	10	21	14.8 \pm 6.6
05MAY01	6	12	15	17	12	28	21.0 \pm 7.0
06MAY01	7	19	10	28	19	25	25.3 \pm 7.9
Mean		6.9	7.1	13.7	9.1	13.4	12.6
STD		6.7	5.0	9.2	5.6	11.3	8.3

BT = Dead bee traps in front of the hives
 LS = Linen sheet in front of the hives
 ED = Evaluation day
 STD (\pm) = Standard deviation

Table 10: Individual results of the evaluations of mortality (numbers of dead bees in the dead bee traps and linen sheets in front of the hives) in the control group

Date	ED	Mortality (numbers of dead bees)					Mean number of dead bees/ colony and day (\pm STD)
		Linen sheets in front of the hives	Hive 1	Hive 2	Hive 3	Hive 4	
		LS	BT	BT	BT	BT	
30APR01	1	2	4	0	5	1	3.0 \pm 2.4
01MAY01	2	6	4	39	15	6	17.5 \pm 16.1
02MAY01	3	5	6	10	18	17	14.0 \pm 5.7
03MAY01	4	12	9	11	38	21	22.8 \pm 13.3
04MAY01	5	14	3	20	29	10	19.0 \pm 11.4
05MAY01	6	19	25	28	36	15	30.8 \pm 8.7
06MAY01	7	18	27	35	62	32	43.5 \pm 15.7
Mean		10.9	11.1	20.4	29.0	14.6	21.5
STD		6.6	10.4	14.3	18.8	10.2	14.8

BT = Dead bee traps in front of the hives
 LS = Linen sheet in front of the hives
 ED = Evaluation day
 STD (\pm) = Standard Deviation

Table 11: Average flight intensity (number of forager bees/tree/minute) in the test substance treatment

Date	ED	Time	Clouding (%)*	Mean flight intensity	STD	Mean flight intensity/day (± STD)
30APR01	1	09:40	10	5.4	1.1	10.4 ± 4.6
		12:55	15	12.2	2.6	
		16:05	30	13.6	4.3	
01MAY01	2	10:20	30	10.0	2	9.9 ± 3.0
		12:55	0	11.0	4.1	
		15:00	15	8.8	2.9	
02MAY01	3	10:20	5	6.0	2.2	7.7 ± 3.2
		16:45	5	9.4	3.4	
03MAY01	4	10:50	80	0.6	0.9	2.4 ± 2.8
		13:55	20	4.2	3	
04MAY01	5	11:15	70	4.2	2.6	4.2 ± 2.6
05MAY01	6	10:10	100	0.4	0.5	0.4 ± 0.5
06MAY01	7	12:20	100	0.2	0.4	0.2 ± 0.4
Mean					5.0	
STD					5.0	

ED = Evaluation day

STD (±) = Standard Deviation

* estimate of % cloud cover at time of assessment

Table 12: Average flight intensity (number of forager bees/tree/minute) in the control

Date	ED	Time	Clouding (%)*	Mean flight intensity	STD	Mean flight intensity/day (± STD)
30APR01	1	10:58	10	4.4	1.5	7.3 ± 3.4
		12:25	10	7.2	2.8	
		15:40	30	10.2	3.0	
01MAY01	2	10:45	30	7.6	2.2	8.7 ± 3.6
		13:20	0	10.4	2.4	
		15:35	15	8.2	5.4	
02MAY01	3	10:50	5	5.0	1.9	4.0 ± 2.2
		17:15	5	3.0	2.2	
03MAY01	4	10:15	80	1.4	1.3	1.4 ± 1.2
		14:30	20	1.4	1.1	
04MAY01	5	10:45	70	3.6	1.1	3.6 ± 1.1
05MAY01	6	10:40	100	0.4	0.5	0.4 ± 0.5
06MAY01	7	12:00	100	0.0	0.0	0.0 ± 0.0
Mean					3.6	
STD					4.1	

ED = Evaluation day
 STD = Standard Deviation
 (%) = estimate of % cloud cover at time of assessment

Table 13: Average flight activity in front of the hives (number of bees entering/leaving the hive/minute) of the test substance treatment

Date	ED	Time	Hive 5		Hive 6		Hive 7		Hive 8		Mean per day and colony (\pm STD)
			Mean	STD	Mean	STD	Mean	STD	Mean	STD	
30APR01	1	09:45	1.00	0.00	0.50	0.71	4.00	2.83	4.50	2.12	16.25 \pm 11.47
	1	12:40	19.00	4.24	20.00	7.07	27.50	0.71	17.50	4.95	
	1	15:55	17.50	3.54	27.00	0.00	28.50	7.78	28.00	14.14	
Mean/day			12.50		15.83		20.00		16.67		
01MAY01	2	10:30	20.50	0.71	26.00	0.00	15.00	4.24	22.50	6.36	32.54 \pm 11.47
	2	12:45	40.50	7.78	23.50	6.36	45.50	3.54	35.00	2.83	
	2	15:10	37.00	1.41	34.50	6.36	50.00	2.83	40.50	13.44	
Mean/day			32.67		28.00		36.83		32.67		
02MAY01	3	10:35	42.50	16.26	43.50	2.12	67.50	28.99	34.50	0.71	48.38 \pm
	3	16:35	49.00	7.07	38.00	1.41	58.00	1.41	54.00	5.66	13.89
Mean/day			45.75		40.75		62.75		44.25		
03MAY01	4	10:55	31.00	29.70	23.50	10.61	31.00	7.07	26.50	0.71	42.31 \pm
	4	13:40	57.50	6.36	50.00	4.24	58.00	0.00	61.00	8.49	17.64
Mean/day			44.25		36.75		44.50		43.75		
04MAY01	5	11:20	66.00	2.83	52.00	2.83	63.50	16.26	63.50	10.61	61.25 \pm 8.87
05MAY01	6	11:15	21.50	6.36	20.00	2.83	29.00	7.07	21.00	2.83	22.88 \pm 5.11
06MAY01	7	12:30	17.00	1.41	8.00	0.00	9.00	2.83	13.00	2.83	11.75 \pm 4.13
Mean/day (\pm STD)			34.24 \pm 19.60		28.76 \pm 15.27		37.94 \pm 21.97		33.55 \pm 18.62		33.62 \pm 19.02

ED = Evaluation day
 STD = Standard Deviation

Table 14: Average flight activity in front of the hives (number of bees entering/leaving the hive/minute) of the control

Date	ED	Time	Hive 1		Hive 2		Hive 3		Hive 4		Mean per day and colony (\pm STD)
			Mean	STD	Mean	STD	Mean	STD	Mean	STD	
30APR01	1	10:05	3.00	1.41	4.00	4.24	5.50	2.12	6.00	1.41	19.88 \pm 11.89
	1	12:15	23.50	2.12	23.00	9.90	30.00	1.41	26.50	0.71	
	1	15:20	29.00	1.41	27.00	7.07	35.50	4.95	25.50	3.54	
Mean/day			18.50		18.00		23.67		19.33		
01MAY01	2	10:50	20.00	2.83	19.50	0.71	18.50	2.12	26.00	7.07	36.13 \pm 13.46
	2	13:10	35.50	0.71	40.00	4.24	40.00	8.49	42.00	14.14	
	2	15:20	36.50	10.61	51.00	2.83	56.00	9.90	48.50	4.95	
Mean/day			30.67		36.83		38.17		38.83		
02MAY01	3	10:50	47.00	4.24	58.50	17.68	57.00	5.66	48.50	4.95	60.00 \pm
	3	17:00	55.00	8.49	76.00	2.83	65.50	4.95	72.50	0.71	11.70
Mean/day			51.00		67.25		61.25		60.50		
03MAY01	4	10:20	27.00	1.41	53.00	15.56	46.00	18.38	60.50	10.61	56.94 \pm
	4	14:10	57.50	9.19	76.00	8.49	58.00	5.66	77.50	9.19	17.57
Mean/day			42.25		64.50		52.00		69.00		
04MAY01	5	10:50	53.50	2.12	70.50	12.02	59.50	3.54	61.00	4.24	61.13 \pm 7.72
05MAY01	6	11:00	14.00	5.66	22.00	5.66	22.00	2.83	21.50	3.54	19.88 \pm 4.70
06MAY01	7	12:10	1.00	1.41	10.50	7.78	3.00	1.41	14.50	0.71	7.25 \pm 6.61
Mean/day (\pm STD)			30.13 \pm 18.83		41.37 \pm 25.37		37.08 \pm 21.33		40.67 \pm 22.75		37.32 \pm 22.25

ED = Evaluation day
 STD = Standard Deviation

Table 15: Brood development of the colonies in the test substance treatment

	Hive 5	Hive 6	Hive 7	Hive 8
1st evaluation: 27APR2001				
Strength (No. of combs covered with bees)	8	7	8	8
No. of combs covered with brood	7	6	7	5
Average amount of egg stage in %	10.00	16.67	12.14	19.00
Average amount of larval stage in %	22.14	24.17	17.14	14.00
Average amount of capped stage in %	26.43	4.17	25.00	36.00
2nd evaluation: 07MAY2001				
Strength (No. of combs covered with bees)	8	7	9	9
No. of combs covered with brood	9	6	9	9
Average amount of egg stage in %	6.11	9.17	17.22	20.56
Average amount of larval stage in %	18.33	21.17	20.56	18.33
Average amount of capped stage in %	43.89	42.50	36.67	35.56
3rd evaluation: 28MAY2001				
Strength (No. of combs covered with bees)	8	8	8	8
No. of combs covered with brood	8	7	9	9
Average amount of egg stage in %	8.13	15.71	12.78	8.33
Average amount of larval stage in %	15.63	20.00	25.56	34.44
Average amount of capped stage in %	41.25	43.57	33.89	28.89

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Table 16: Brood development of the colonies in the control

	Hive 1	Hive 2	Hive 3	Hive 4
1st evaluation: 27APR2001				
Strength (No. of combs covered with bees)	7	7	7	8
No. of combs covered with brood	6	8	7	7
Average amount of egg stage in %	15.83	10.63	16.43	11.43
Average amount of larval stage in %	10.00	12.50	22.86	25.71
Average amount of capped stage in %	10.83	26.88	10.71	20.71
2nd evaluation: 07MAY2001				
Strength (No. of combs covered with bees)	7	8	7	9
No. of combs covered with brood	6	9	7	9
Average amount of egg stage in %	4.17	8.33	9.29	10.00
Average amount of larval stage in %	9.17	15.00	14.29	20.00
Average amount of capped stage in %	40.00	42.78	38.57	44.44
3rd evaluation: 28MAY2001				
Strength (No. of combs covered with bees)	7	8	6	8
No. of combs covered with brood	0	9	2	10
Average amount of egg stage in %	0.00	7.78	0.00	7.00
Average amount of larval stage in %	0.00	23.33	0.00	14.50
Average amount of capped stage in %	0.00	41.67	10.00	39.50

Table 17: Number of bees with apple pollen loads entering the hives of the test substance treatment

Date	ED	Time	Hive	Total number of bees with pollen loads	Number of bees with apple pollen loads	Percentage of bees with apple pollen loads
01MAY01	2	13:00	7	16	16	100.0
			8	18	18	100.0
		15:10	5	12	12	100.0
			6	11	11	100.0
03MAY01	4	11:00	6	18	2	11.1
			7	11	11	100.0
		13:50	7	16	13	81.3
			8	19	15	78.9

ED = Evaluation day

Table 18: Number of bees with apple pollen loads entering the hives of the control treatment

Date	ED	Time	Hive	Total number of bees with pollen loads	Number of bees with apple pollen loads	Percentage of bees with apple pollen loads
01MAY01	2	13:15	3	16	14	87.5
			4	15	14	93.3
		15:20	1	8	7	87.5
			2	14	14	100.0
03MAY01	4	10:30	2	14	13	92.9
			3	13	12	92.3
		14:15	1	17	15	88.2
			4	20	18	90.0

ED = Evaluation day

Table 19: Results of recording of the flowering stages in the fields

Date	ED	BBCH code of the apple flowers	
		Test substance field	Control field
29APR01	0	64 – 65	64 – 65
01MAY01	2	65	64 – 65
03MAY01	4	65	65
05MAY01	6	65	65

ED: Evaluation day after the beginning of bee exposure

*Remark:

BBCH codes	Explanation
64	40 % of flowers open
65	Full flowering 50 % of flowers open, older petals falling

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Table 20: Results of the evaluation of the fruit buds in the test substance treated field

Date	ED	Spot No.	Buds [%]	Flowers in [%]	Fruit buds [%]	Ø Fruit buds [%]
05MAY01	6	1	2.7	4.1	93.2	93.1
		2	1.4	11.1	87.5	
		3	0.0	3.9	96.1	
		4	1.4	5.4	93.2	
		5	0.0	4.5	95.5	
14MAY01	15	1	0.0	0.0	87.8	89.1
		2	0.0	0.0	98.6	
		3	0.0	0.0	85.5	
		4	0.0	0.0	98.6	
		5	0.0	0.0	75.0	
12JUN01	44	1	0.0	0.0	5.4	4.6
		2	0.0	0.0	1.4	
		3	0.0	0.0	7.9	
		4	0.0	0.0	1.4	
		5	0.0	0.0	6.8	

ED = Evaluation day

Remark: The sum of buds, flowers and fruit buds observed at the first assessment on ED 6 is 100%

Table 21: Results of the evaluation of the fruit buds in the control field

Date	ED	Spot No.	Buds [%]	Flowers in [%]	Fruit buds [%]	Ø Fruit buds [%]
05MAY01	6	1	0.0	1.4	98.6	93.6
		2	1.2	3.6	95.2	
		3	0.0	0.0	100.0	
		4	1.4	1.4	97.1	
		5	0.0	23.1	76.9	
14MAY01	15	1	0.0	0.0	77.1	83.4
		2	0.0	0.0	96.4	
		3	0.0	0.0	81.7	
		4	0.0	0.0	67.7	
		5	0.0	0.0	94.9	
12JUN01	44	1	0.0	0.0	18.6	12.0
		2	0.0	0.0	10.8	
		3	0.0	0.0	19.7	
		4	0.0	0.0	4.3	
		5	0.0	0.0	6.4	

ED = Evaluation day

Remark: The sum of buds, flowers and fruit buds observed at the first assessment on ED 6 is 100 %

Table 22: Total weights of the test colonies 5 to 7 in the Confidor SL 200 treatment recorded once a day on a beam-scale

Weights of the Confidor SL 200 colonies [kg]				
Date	ED	Hive 5	Hive 6	Hive 7
29APR01	0	39.7	36.7	44.3
30APR01	1	38.7	36.0	43.3
01MAY01	2	38.7	35.8	42.9
02MAY01	3	39.5	36.1	43.2
03MAY01	4	39.8	36.1	43.1
04MAY01	5	40.5	36.1	43.7
05MAY01	6	40.4	36.0	43.5
06MAY01	7	40.3	36.1	43.4
Weight gain/loss [%]		+1.5	-1.6	-2.0

Remark: ED = Evaluation day

Table 23: Total weights of the test colonies 1 to 3 in the control treatment recorded once a day on a beam-scale

Weights of the control colonies [kg]				
Date	ED	Hive 1	Hive 2	Hive 3
29APR01	0	38.0	43.3	39.3
30APR01	1	37.0	41.8	38.5
01MAY01	2	36.7	42.2	38.2
02MAY01	3	37.0	44.1	38.3
03MAY01	4	36.8	44.0	38.2
04MAY01	5	36.8	44.5	38.0
05MAY01	6	36.5	44.2	37.8
06MAY01	7	36.3	44.0	37.7
Weight gain/loss [%]		-4.5	+1.6	-4.1

Remark: ED = Evaluation day

A3 Certificates

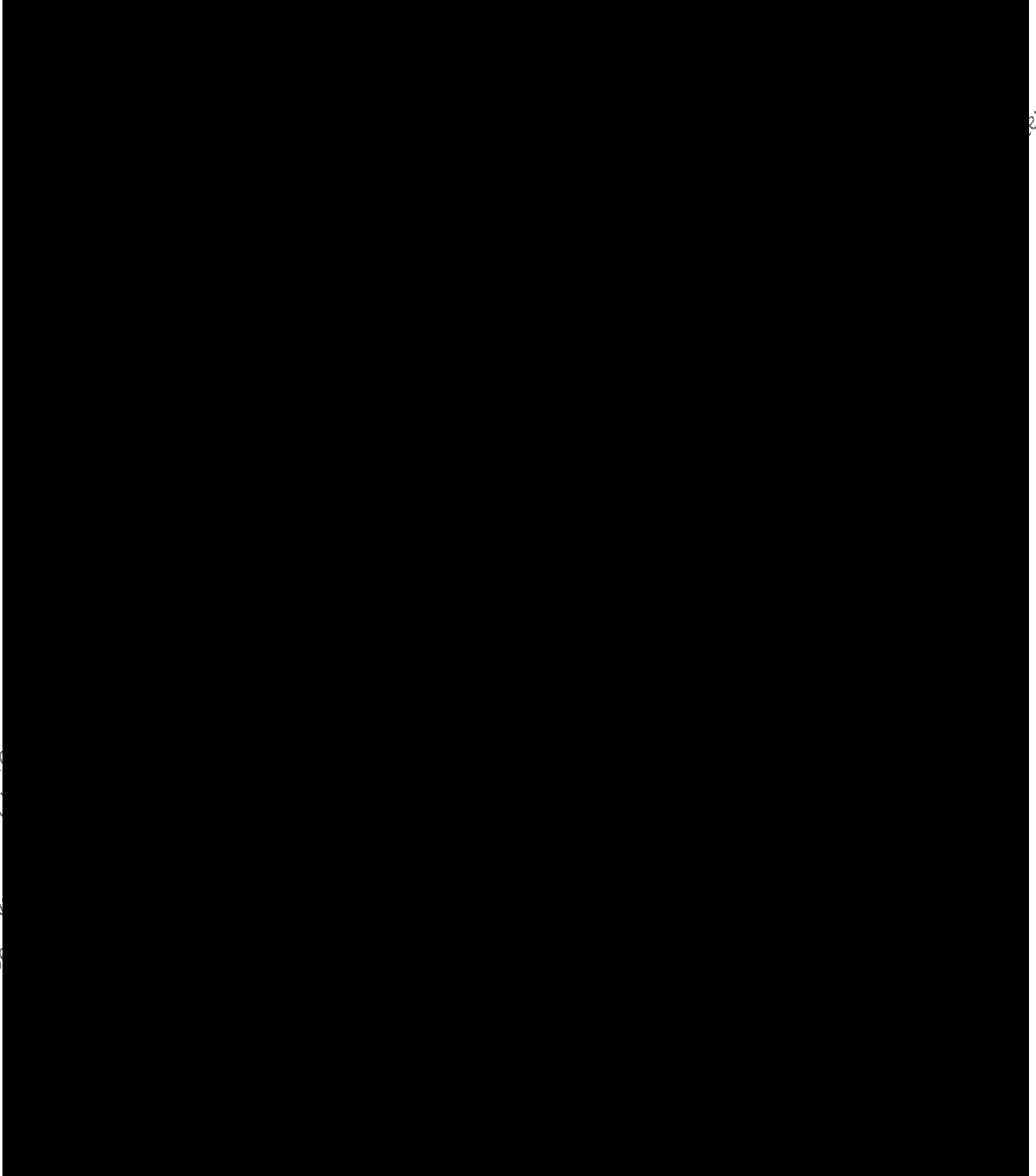


Figure 5: Certificate of analysis of Confidor SL 200

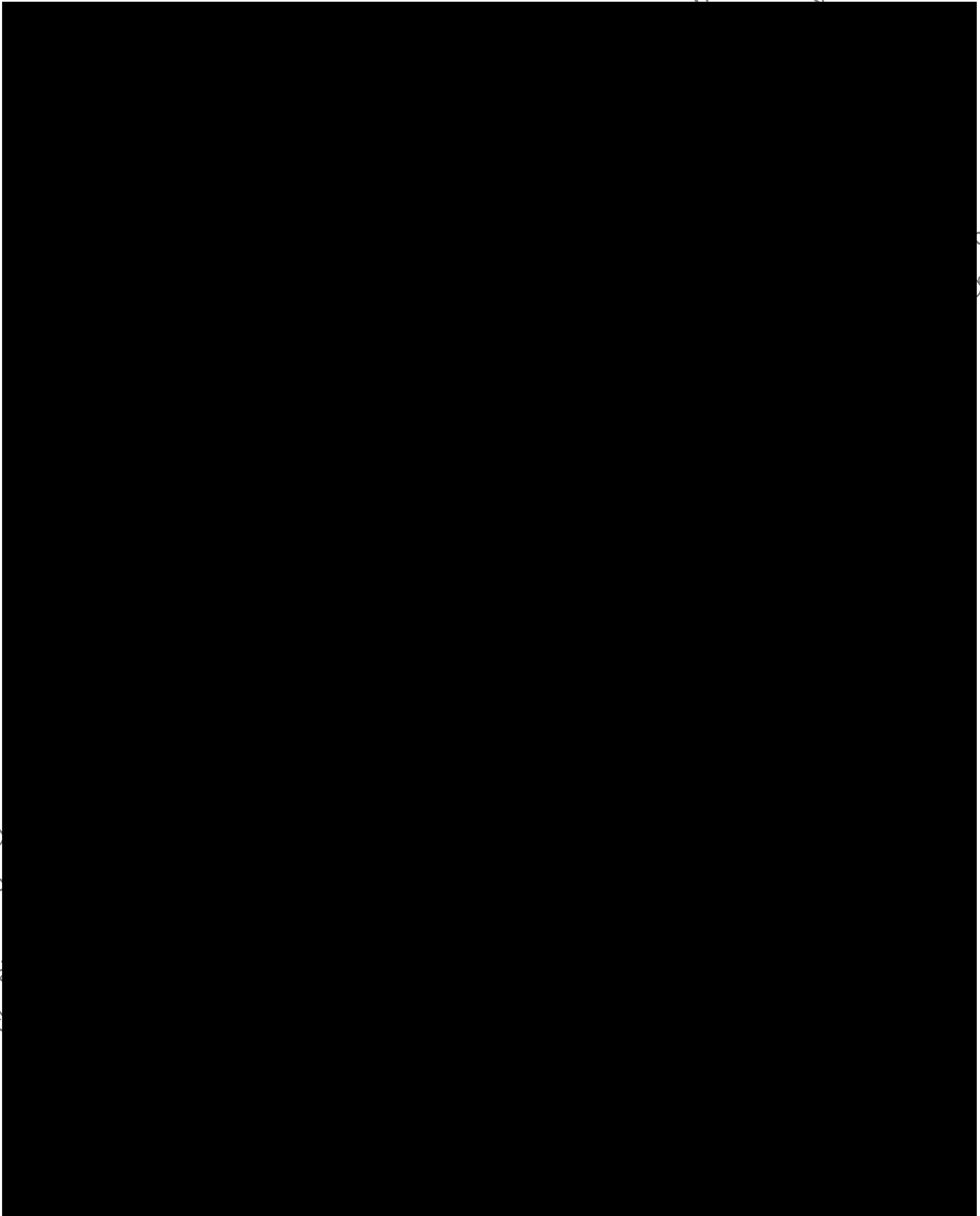


Figure 6: GLP Certificate of testing facility