Final Report

Field Test: Side Effects of Oil-Seed Rape grown from Seeds Dressed with Imidacloprid and Beta-Cyfluthrin FS 500 on the Honey Bee (*Apis mellifera* L.)

Guideline

EPPO 170

Study Director

[redacted]

Date

06/06/2002

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Study Identification Code

Test substance: Imidacloprid & Beta-Cyfluthrin FS 500
Study code: 99398/01-BFEU

99398/01-BFEU / MO-02-007981

Statement of Confidentiality

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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, Federal Republic of 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 basis of intergovernmental agreements.

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Statement of Quality Assurance Unit

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Quality assurance
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1 Summary

Report: [redacted] Field Test: Side Effects of Oil-Seed Rape grown from Seeds Dressed with Imidacloprid and Beta-Cyfluthrin FS 500 on the Honey Bee (*Apis mellifera* L.)

Source: Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, unpublished report No: 99398/01-BFEU, issued 06/06/2002.

Guidelines: EPPO guideline No. 170: Guideline on test methods for evaluation the side-effects of plant protection products on honey bees (EPPO, 1992) and the SETAC guideline: Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, Point 5.3.
Deviations: Yes, but without impact on the study results.

GLP: Yes (certified laboratory)

Procedures

Materials and methods:

Fields with oil-seed rape (*Brassica napus*, variety Lirajet) dressed with 1051.17 g a.i. & 187.31 g a.i./100 kg seeds Imidacloprid & Beta-Cyfluthrin FS 500 (dressed seeds: article number 02 00944819 A, product used for dressing: development number 0195939, formulation number 0055, tox number 4867-00) and the fungicide Thiram were used as test substance treatment group. Plots with oil-seed rape dressed only with Thiram served as control.

The effect of the test substance was examined on bee colonies placed next to the fields at the begin of the full flowering stage of *Brassica napus* L. The study was carried out with one replicate (one field) per treatment group. Two groups of three hives were placed next to each field. One group served as test colonies, the other for the collection of nectar, pollen and honey. The bees were exposed to the flowering oil-seed rape from the 27/04/2000 until the 12/05/2000 (BBCH 61-62, start of blooming until BBCH 69, end of flowering).

From the 28/04/2000 until the 11/05/2000 mortality and foraging activity of the bees were assessed once a day. The strength of the colonies and the development of the bee brood were assessed 4 times during the

study. Additionally the weight from the bee hives of the first group was recorded continuously.

Samples of pollen, nectar and honey were collected during the study, for analysis of residues of the test substance and metabolites of the test substance.

The influence of the test substance Imidacloprid & Beta-Cyfluthrin FS 500 was evaluated by comparing the bees of the test field to the bees of the control field.

Dates of work: 23/08/1999 – 13/06/2000

Biological Findings:

Test substance	Imidacloprid & Beta-Cyfluthrin FS 500			
Test organism	*Apis mellifera*			
Exposure	Oil-seed rape			
Endpoints	Control field		Test substance field	
Dead bees in the bee traps and in front of bee hives	504		350	
Dead bees in the field	2		11	
Mean flight activity	2.3 bees/m^2/min		3.3 bees/m^2/min	
Colony strength described by the weight of the test colonies	hive 76	+ 31.7 kg (54.7 %)	hive 35	+ 24.3 kg (44.6 %)
	hive 90	+ 26.3 kg (49.0 %)	hive 124	+ 27.7 kg (52.5 %)
	hive 15	+ 24.8 kg (44.5 %)	hive 19	+ 24.4 kg (47.6 %)

Analytical Findings:

Test substance	Imidacloprid & Beta-Cyfluthrin FS 500					
Test organism	*Apis mellifera*					
Exposure	Oil-seed rape					
Sample material	Control field			Test substance field		
Analysed for [mg/kg]	Hydroxy-Imida-cloprid	Olefin-Imida-cloprid	Imida-cloprid	Hydroxy-Imida-cloprid	Olefin-Imida-cloprid	Imida-cloprid
Nectar from the comb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d. to < LOQ
Pollen from the comb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Honey from the comb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nectar from the blossoms	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Limit of quantitation: 0.005 mg/kg for Imidacloprid and Hydroxy-Metabolite, 0.01 mg/kg for the Olefin-Metabolite
< 0.005 and < 0.010 = Residues below the limit of quantitation (< LOQ)
Limit of detection: 0.0015 mg/kg for Imidacloprid and Hydroxy-Metabolite, 0.003 mg/kg for the Olefin-Metabolite
n.d.: Residues below the limit of detection

Observations:

There were no adverse affects of the treatment on foraging activities of the bees, colony weight and development or mortality. No behavioural impacts (e.g. apathy, exaggerated motility, discoordinated movements) were observed on the honey bees collecting rape, nectar and pollen on the test substance field compared to the control. The development of bee brood was not affected by the test substance and was nearly similar in the hives exposed to the test substance field or to the control field.
Likewise, in the analytical part of this study no residues of metabolites of the test substance were found in pollen, nectar or honey. In the nectar collected out of the combs residues of the test substance below the limit of quantitation (< 0.005 mg/kg) were found. In the other samples (pollen and honey from the combs and nectar from the blossoms) no residues of the test substance were found.

2 Time Schedule

Study initiation date:	01/10/1999
Start of the experimental phase:	23/08/1999
End of the experimental phase:	13/06/2000
Draft report:	24/10/2001
Final report:	06/06/2002

3 Study Objective

The objective of this study was to determine the effects of oil-seed rape dressed with Imidacloprid & Beta-Cyfluthrin FS 500 on the honey bee *Apis mellifera* (Hymenoptera, Apidae) under field conditions following the EPPO guideline No. 170 (EPPO, 1992) and the SETAC guideline (1995).

4 Material and Methods

4.1 Test Substance

Name:	Imidacloprid & Beta-Cyfluthrin FS 500
Development number:	0195932
Formulation number:	0055
Tox number:	4867-00
Common names:	a) Imidacloprid b) Beta-Cyfluthrin
Active ingredients:	a) NTN 33893 b) FCR 4545
CAS number:	a) 138261-41-3 b) 68359-37-5
Intended use:	Insecticide
Contents of a.i. nominal:	a) 420 g/L b) 80 g/L
Contents of a.i. analysed:	a) 433.53 g/L b) 77.25 g/L
Date of analysis:	03/03/1999
Expiry date:	03/09/1999
Appearance/colour:	purple/fluid

Density:	1.153 g/mL
Storage conditions:	The test material was stored dark and dry at room temperature (> 5 °C, < 25 °C)
Safety precautions:	General precautions for handling of test substances.

4.2 Dressed Seeds

Name:	Oil-seed rape (variety: Lirajet) dressed with Imidacloprid & Beta-Cyfluthrin FS 500 and with a fungicide as stated in chapter 4.4
Treated seed identification (Bay):	02 00944819 A
GAB No. of dressed seeds:	99398
Loading rate (nominal):	1050 g a.i. imidacloprid/100 kg seeds
Loading rate measured:	1051.17 g a.i. imidacloprid/100 kg seeds
Expiry date:	03/09/1999
Safety precautions:	General precautions for handling of test substances.
Storage conditions:	The test material was stored dark and dry at room temperature (> 5 °C, < 25 °C)

4.3 Control treatment group

Oil-seed rape (variety: Lirajet) (GAB No. of control seeds 99397), only dressed with a fungicide as stated in chapter 4.4.

4.4 Additional Seed Treatments

Both, control and test substance seeds were additionally dressed with Thiram (362.59 g a.i./100 kg seeds), a fungicide which is known to have no insecticide activity under the test conditions.

Name:	Thiram
VSE number:	00 04403592 00
PRE number:	02 00944827
CAS number:	137-26-8
Intended use:	Fungicide

Storage conditions:	The test material was stored dark and dry at room temperature (> 5 °C, < 25 °C)
Safety precautions:	General precautions for handling of test substances.

4.5 Principles of the Study

The field study was carried out according to the EPPO guideline No. 170: Guideline on test methods for evaluation the side-effects of plant protection products on honey bees (EPPO, 1992) and the SETAC guideline: Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, Point 5.3. (1995).
One field with oil-seed rape (*Brassica napus* L.) dressed with Imidacloprid & Beta-Cyfluthrin FS 500 was used for the test substance treatment group (size approximately 23432 m^2, dressed with 1051.17 g a.i. imidacloprid /100 kg seeds and sown with 7.0 kg seeds/23432 m^2). A second field (25919 m^2) sown with 8.4 kg seeds/25919 m^2 of untreated oil-seed rape served as the control treatment group.

The effect of the test substance was examined on honey bee colonies which were placed in groups of six hives next to each field prior to the full flowering stage of *Brassica napus* L.

Mortality, foraging activity and the condition of the colonies were assessed from 28/04/2000 until the 11/05/2000. The development of the bee brood was assessed 4 times from the 02/05/2000 until the 13/06/2000.

Additionally, samples of pollen, nectar and honey were collected from the hives during the study, for the purpose of subsequent chemical analysis.

After the end of flowering (12/05/2000) all bee hives were transported to the garden of the Niedersächsisches Landesinstitut für Bienenkunde on 27/06/2000 (about 40 km away from the test fields). The colonies were maintained there until the end of the bee season.

The influence of the test substance Imidacloprid & Beta-Cyfluthrin FS 500 was evaluated by comparing the parameters assessed that are mentioned above, in the test substance treatment group to those in the control treatment group.

4.6 Description of the Test Method

4.6.1 Test Location and Test Plant

The test fields were located in the north of Germany near Hetendorf (Niedersachsen). The sowing was carried out on 23/08/1999. The size of the field covered with *Brassica napus* dressed with Imidacloprid & Beta-Cyfluthrin FS 500 was 23432 m^2 and the control field was about 25919 m^2. The distances between these fields was at least 4 km. Around the fields no other flowering plants were grown. In the nearby surroundings potatoes and cereals were grown. In the area of the test fields no other bee hives were placed. In the last few years no Imidacloprid was used as insecticide on the fields used in this study. Details about sowing are given in Table 1 and on a map with the test area in Figure 6 to Figure 9 in Appendix 7.

Table 1: Details of the sowing regime

Date		23.08.1999
Drilling machine		Amazone 07, Typ 30
Sowing rate control field*		8.4 kg/25919 m^2
Sowing rate test substance field*		7.0 kg/23432 m^2
Soil temperature in 20 cm depth in the control field	°C	11
Soil temperature in 20 cm depth in the test substance field	°C	11

*Before sowing the weight of the seeds that were filled into the seeding machine was determined. After sowing of the field, the remaining seeds were weight. The difference between these two weights is the sowing rate.

First the control field (seeds were dressed only with Thiram) and then the test substance field was sown, no cleaning of the drilling machine was necessary. No other plant protection products were used in that season.

4.6.2 Experimental Bee Colonies

Six normally developed, queen-right colonies, which were as similar as possible in size and strength, (Zandermaß) (*Apis mellifera carnica* L.) were used per treatment group. For each of the colonies used a certificate of health was obtained from the national authorities (responsible veterinarian) prior to test start. Each colony contained 2 brood bodies with 6-8 brood frames and 1 honey body with at least 8 frames. The bees for the study were supplied by the Niedersächsisches Landesinstitut für Bienenkunde, Wehlstr. 4a, D-29221 Celle, Germany.

4.6.3 Set-up of the Bee Colonies on the Test and Control Sites

At the beginning of flowering (BBCH-code: 61-62: start of blooming, ligulate blossoms vertical on the disc, tubiform blossoms visible in the furthest third), six bee colonies each were set-up at the edge of the test field (see Appendix 7, Figure 7) and the control field (see Appendix 7, Figure 9). Always three of these hives were placed side by side (1st colony group) with dead bee traps attached to the hive entrances over the whole test period. The bee traps (wooden bee traps, width 38 cm, depth 25 cm and height 22 cm) were covered with gauze (mesh size 0.5 cm x 0.5 cm) on bottom and 50 % on the top. Bees entering and leaving the hive had to pass through the mesh, the size of which allowed normal bee movements but prevented bees from being able to pass through whilst carrying dead bees removed from the hive. Such dead bees were collected within the trap. Photos of the bee traps, hives and linen sheets are given in the Appendix 6. Additionally, water-permeable linen sheets (each covering an area of 5 m^2) were laid out in front of the hives and also on three different places in the test fields (the linen sheets in the field were covering an area of in total 15 m^2) to record the number of dead honey bees. The linen sheets were distributed over the fields in such a way, that in every flowering stage of the oil-seed rape a linen sheet was placed. The locations of the linen sheets within the field are indicated in Appendix 7.

The other 3 colonies (2nd colony group) were set-up at a distance of at least 30 m to the hives equipped with dead bee traps. These second groups of hives in control and treatment were only used for the collection of pollen and honey.

Table 2: Hive numbers of the different colonies used

	Control field		Test substance field	
	1st colony group	2nd colony group	1st colony group	2nd colony group
No of hives	76	72	19	126
	90	86	124	69
	15	114	35	108

4.6.4 Recording of the Stages of Blooming of the Plants

The stages of flowering of the oil-seed rape plants were recorded all 3 days (BBCH-code) and are documented in the raw data and in Appendix 5.

4.6.5 Recording of the Meteorological Data

For the entire test period, the following weather data were recorded:

- temperature
- relative humidity
- rainfall
- bad weather conditions and their approximate duration (for example storms, hail)

During the study a calibrated data-logger (ESCORT Messtechnik AG, CH-8904 Aesch) and a rain gauge standing beside the fields recorded the meteorological data.

4.7 Mode of Assessment

4.7.1 Mortality

On the day after setting up of the colonies at the edge of the fields (beginning of the full flowering, ligulate blossoms vertical on the disc, tubiform blossoms visible in the furthest third, 27/04/2000), the hives of the first colony groups of the control and treatment were equipped with traps for collection of dead individuals. Observation of mortality started on the next day. The number of dead bees found in front of the hives on the linen sheet and in the dead bee traps was recorded and the dead bees were removed once per day for a period of 14 days (28/04/2000-11/05/2000). Mortality assessments were not carried out in the second colony groups.

For the recording of the number of dead bees in each treatment group in the field, all plant material was removed from three individual spots (20/04/2000), and the ground was covered with water-permeable linen sheets (approximately 15 m^2, 0.5 x 10 m each). Because not all oil seed plants on the field were at the same flowering stage, the spots were distributed over the field including different developmental stages of the flowering rape plants. The locations of these linen sheets in control and treatment within the fields are indicated in Appendix 7, Figure 7 and 9. The dead bees on the linen sheets were recorded and

removed once per day for a period of 14 days (28/04/2000-11/05/2000).

4.7.2 Flight Intensity of the Bees in the Field

The observations of the flight intensity in the field started one day after the set-up of the hives at the time of the start of full bloom (28/04/2000) (BBCH-code: 61-62: start of blooming, ligulate blossoms vertical on the disc, tubiform blossoms visible in the furthest third) of the oil-seed rape plants and took place in five marked squares in each treatment group (each 1 m^2). Squares were marked and distributed over the field to cover different developmental stages of the flowering rape. At each assessment the same squares were used. At each assessment time the number of bees that were either foraging on flowering rape plants or flying close over the crop were counted instantaneously for a period of one minute.

Assessments of the flight intensity were carried out from the beginning of the start of full flowering up to three times a day, for a period of 14 days (28/04/2000-11/05/2000). The times of the assessments and the weather conditions at the time of the assessments (temperature, relative humidity and an estimate of % cloud cover) were recorded and are documented in the Appendix section of this report (28/04/2000-11/05/2000).

4.7.3 Condition of the Colonies and Development of the Bee Brood

The condition of the colonies and the development of the bee brood were checked every 6 (±1) days during the complete flowering period of the oil-seed rape (28/04/2000-11/05/2000). The first check was performed five days after the set-up of the hives.
The last check of the colonies was carried out 33 days after the end of the period of flowering. Assessments were carried out with all of the 6 colonies.

During each observation, the following parameters were assessed:

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

Brood assessments were performed according to beekeeper practice by experienced personnel. The comb area containing eggs, larvae, pupae and pollen and nectar were estimated and classified as small

(means below expected development status), medium (means expected development status), good (means better than expected development status) and very good (means much better than expected development status).

4.7.4 Weight of the Test Colonies

The weight development (increase or decrease) of the three colonies of the 1st group was recorded continuously once per day from the 27/04/2000 until the 15/05/2000 using a beam scale (Zeidler-Memo-Waage, supplier Simone Raff, Pestalottzistr. 24, 76351 Linkenheim-Ho, Germany). In order to prevent rainfall directly influencing the results of the weight assessments of the hives, rain proof protection covered all of the hives.

The second groups of hives were only used for the collection of pollen and honey.

4.7.5 Collection of Honey and Pollen from the Test Colonies

During the first three assessments of the brood of the colonies of the 2nd group of each treatment group, one old honey comb filled with rape nectar per hive was extracted (02/05/2000, 08/05/2000 and 15/05/2000) and stored in a plastic bag at approximately - 20° C for the purpose of a subsequent residue analysis. An empty comb substituted the extracted one. Additionally the honey of the 1st colony groups was extracted (about 50 kg with a honey extractor) on the 22/05/2000 and stored in a plastic bag at approximately - 20° C but was not subjected to a subsequent analysis.

For the determination of pollen brought into the hives bee bread (areas in the combs with a high pollen amount) was cut out of the combs of the 2nd group on the 02, 05, 08 and 15/05/2000.

The bee bread was collected and stored in plastic pots at approximately - 20° C for the purpose of a subsequent residue analysis. The results of the residue analysis are given in Table 9 and Appendix 10.

4.7.6 Behaviour of the Bees

During the assessment period of 14 days, the behaviour of the bees in front of the hive and in the field was observed. Particularly abnormal or unusual behaviour (e.g. way of collection, latency behaviour, behaviour when leaving or entering the hive, defence behaviour at the access hole) was recorded.

4.8 Further assessments

4.8.1 Collection of Nectar directly from the rape blossoms

For the purpose of subsequent analysis, nectar was taken out of the rape blossoms with micro-capillaries. To prevent that insects will collect nectar from the blossoms small parts of the fields were covered with tents.

The results of the residue analysis carried out on 04/05/2000 are given in Table 9 and Appendix 10.

4.8.2 Collection of Dead Bees

Following each relevant assessment time point, the dead bees from the wooden bee traps and from the water-permeable linen sheets were collected and stored in plastic bags at approximately - 20° C for the purpose of possible subsequent residue analysis which was not carried out.

4.9 Evaluation of the Test Results

The influence of the test substance Imidacloprid & Beta-Cyfluthrin FS 500 was evaluated by comparing the bees of the different treatment groups in view of the following observations:

- Mortality in front of the bee hives, in the dead bee traps and on the linen sheets laid out in the fields.
- Foraging activity:
  Number of forager bees/m² flowering *Brassica napus* crop/minute
  (Attractiveness of Imidacloprid & Beta-Cyfluthrin FS 500 dressed crop in comparison to the untreated (control) crop.
- Behaviour of the bees on the crop and around the hive.
- Development of the bee brood.
- Weight development of the hives.

4.10 Residue Analysis of samples

All samples collected were sent deep frozen to the Bayer AG (Research Centre Monheim, Institute for Ecobiology, D-51368 Leverkusen, Germany) for analysis.

The results of the residue analysis are given in Table 9 and Appendix 10.

5 Deviations from the Study Plan

The study was performed according to the study plan dated 22/09/1999, the amendment No. 1 dated 05/04/2000, the amendment No. 2 dated 11/04/2002 and the following deviations:

1. Mode of Assessment
   Deviation: The flight intensity assessments of both treatment groups were performed four times per day on the 03, 04, 05 and 08/05/2000 and not only once a day as stated in the study plan.
   Reason: The observations were repeated to detect any possible effects during times with a high flight intensity.
   Impact on the study: None.

2. Conditions of the colonies and development of the bee brood
   Deviation: The first check of the bee brood was performed 5 days after the set-up of the hives and subsequently every 6 ± 1 days later and not all 9 ± 1 days as stated in the study plan.
   Reason: To minimise the risk of swarming, which normally occurs in spring time.
   Impact on the study: None.

3. Mortality assessments
   Deviation: On the 03/05/2000 and on the 04/05/2000 the mortality assessment were performed two times per day.
   Reason: After the first brood assessment it was possible that a lot of bees died and the counting of the dead bees would have taken to much time. To prevent this, the mortality assessments were performed two times per day.
   Impact on the study: None.

4. The gaining of bee bread
   Deviation In addition to the brood assessment bee bread was also taken on the 05/05/2000.

Reason: To get a high amount of rape pollen for analysis.
Impact on the study: None.

This report reflects the conduct of this study.

6 Deviations from the guideline

None

7 Time Schedule of the Test

The important dates of the trial are given in Table 3.

Table 3: Dates of the field trial

Activity	DAE	Date
Set-up of test hives	0	27/04/2000
1st evaluation of mortality and flight intensity	1	28/04/2000
1st brood assessment	5	02/05/2000
2nd brood assessment	11	08/05/2000
Last evaluation of mortality and flight intensity	14	11/05/2000
3rd brood assessment	18	15/05/2000
Last brood assessment	47	13/06/2000

Remark: DAE = days after begin of exposure

In the following tables (Table 4 and Table 5) the dates of all the assessments and activities carried out during the field phase are summarised.

Table 4: Assessments carried out during the field phase in the Imidacloprid & Beta-Cyfluthrin FS 500 treatment group

Activity	DAE	Date
Set-up of test hives	0	27/04/2000
Assessment of flight intensity, recording of dead bees	1	28/04/2000
Assessment of flight intensity, recording of dead bees	2	29/04/2000
Assessment of flight intensity, recording of dead bees	3	30/04/2000
Assessment of flight intensity, recording of dead bees	4	01/05/2000
Assessment of flight intensity, recording of dead bees, assessment of brood, honey combs and bee bread were taken from hive 126, 69 and 108	5	02/05/2000
Assessment of flight intensity, recording of dead bees	6	03/05/2000
Assessment of flight intensity, recording of dead bees	7	04/05/2000
Assessment of flight intensity, recording of dead bees, bee bread were taken from hives 126, 69 and 108	8	05/05/2000
Assessment of flight intensity, recording of dead bees	9	06/05/2000
Assessment of flight intensity, recording of dead bees	10	07/05/2000
Assessment of flight intensity, recording of dead bees, assessment of brood, honey combs and bee bread were taken from hive 126, 69 and 108	11	08/05/2000
Assessment of flight intensity, recording of dead bees	12	09/05/2000
Assessment of flight intensity, recording of dead bees	13	10/05/2000
Last assessment of flight intensity, recording of dead bees	14	11/05/2000
Assessment of brood, honey combs and bee bread were taken from hive 126, 69 and 108	18	15/05/2000
Extraction of honey from hive 35, 124 and 19	25	22/05/2000
Last assessment of brood	47	13/06/2000

Remark: DAE = days after begin of exposure

Table 5: Assessments carried out during the field phase in the control group

Activity	DAE	Date
Set-up of test hives	0	27/04/2000
Assessment of flight intensity, recording of dead bees	1	28/04/2000
Assessment of flight intensity, recording of dead bees	2	29/04/2000
Assessment of flight intensity, recording of dead bees	3	30/04/2000
Assessment of flight intensity, recording of dead bees	4	01/05/2000
Assessment of flight intensity, recording of dead bees, assessment of brood, honey combs and bee bread were taken from hive 72, 86 and 114	5	02/05/2000
Assessment of flight intensity, recording of dead bees	6	03/05/2000
Assessment of flight intensity, recording of dead bees	7	04/05/2000
Assessment of flight intensity, recording of dead bees, bee bread were taken from hives 72, 86 and 114	8	05/05/2000
Assessment of flight intensity, recording of dead bees	9	06/05/2000
Assessment of flight intensity, recording of dead bees	10	07/05/2000
Assessment of flight intensity, recording of dead bees, assessment of brood, honey combs and bee bread were taken from hive 72, 86 and 114	11	08/05/2000
Assessment of flight intensity, recording of dead bees	12	09/05/2000
Assessment of flight intensity, recording of dead bees	13	10/05/2000
Last assessment of flight intensity, recording of dead bees	14	11/05/2000
Assessment of brood, honey combs and bee bread were taken from hive 72, 86 and 114	18	15/05/2000
Extraction of honey from hive 15, 76 and 90	25	22/05/2000
Last assessment of brood	47	13/06/2000

Remark: DAE = days after begin of exposure

8 Results

8.1 Mortality

Figure 1 shows the average mortality in the test substance, i.e. oil-seed rape dressed with Imidacloprid & Beta-Cyfluthrin FS 500 and control group (see Table 11 and 12 for more detailed results).

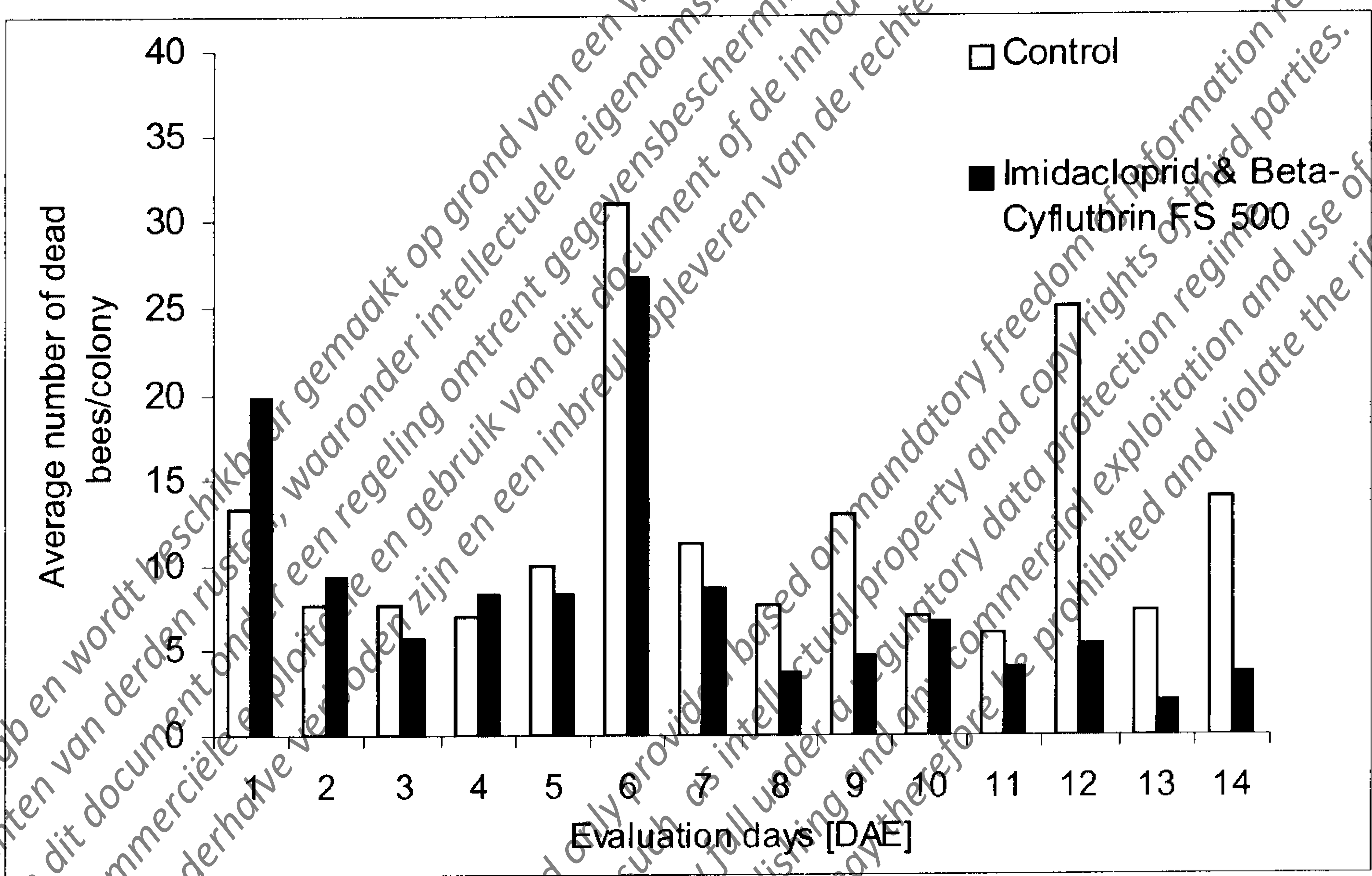


Figure 1: Average number of dead bees collected in the dead bee traps of the first colony group and on the water permeable linen sheets in front of the bee hives in the Imidacloprid & Beta-Cyfluthrin FS 500 and in the control treatment group

The mortality (dead bees in the bee trap and on the linen sheet in front of the hives per colony of the first colony group and day) in the test substance treatment group was only slightly increased on DAE 1, DAE 2, and DAE 4 in comparison to the control treatment group. On the other days (DAE 3 and DAE 5-14) the mortality in the test substance treatment groups was equal or less than the one of the control group. During the evaluation period the daily average mortality ranged from 2.0 dead bees/hive to a maximum of 26.7 dead bees/hive in the test substance treatment group. In the control treatment group the daily average mortality ranged from 6.0 dead bees/hive to 31.0 dead bees/hive. The average mortality during the evaluation days DAE 1 - DAE 14 was 8.3 dead bees/colony and day in the Imidacloprid & Beta-Cyfluthrin FS 500 treatment group, and 12.0 dead bees/colony and day in the control treatment group. The mortality rates of both

treatment groups were in the range of normal mortality and in general very low.
Statistical analysis was not performed with the mortality data, because every hive is a complex and closed biological system and it is not possible to define the number of bees per hive (population), to which these mortality data belong.

8.2 Flight Intensity

Figure 2 shows the average flight intensity in the test substance and control treatment group (see also Table 13 and 14).

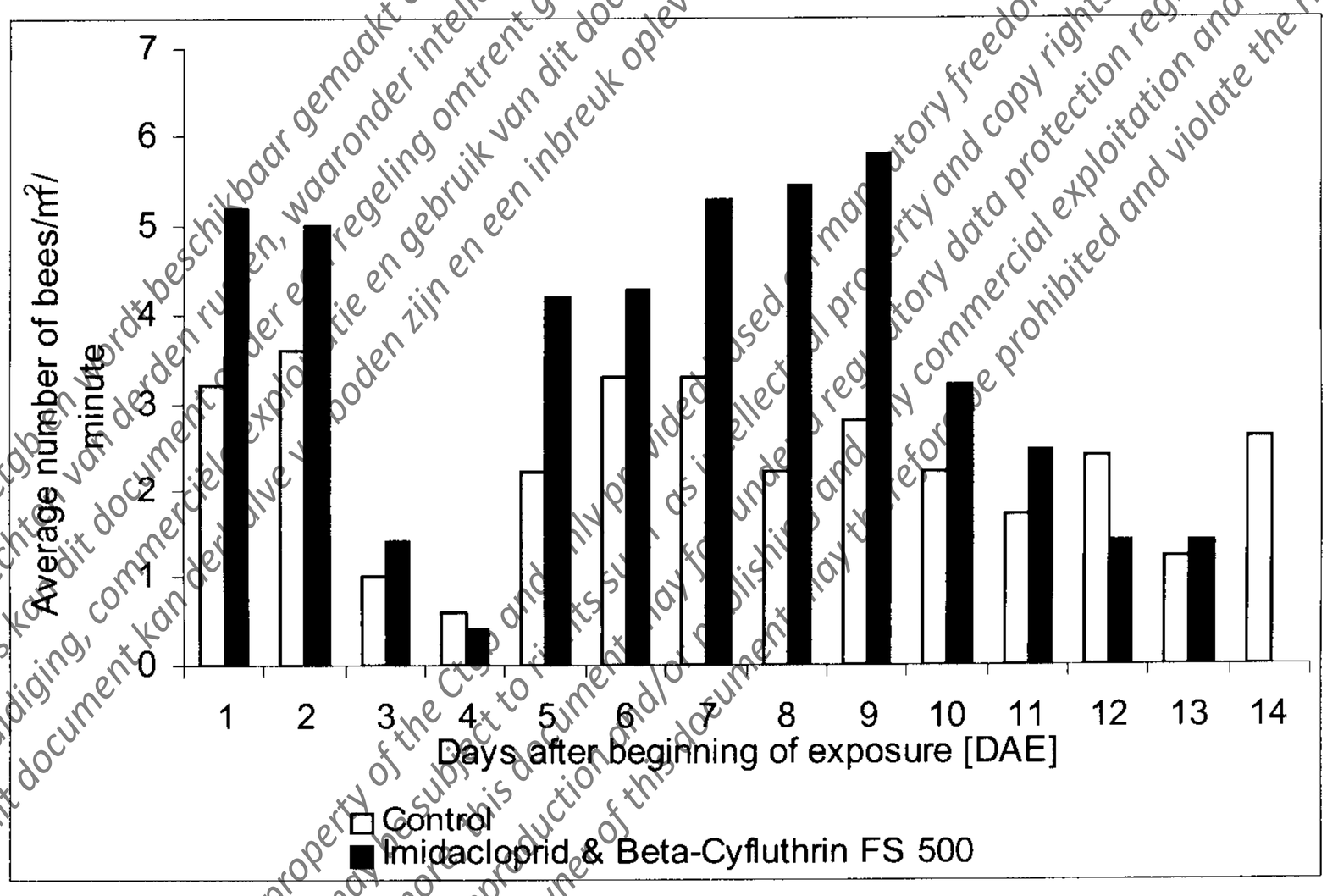


Figure 2: Average flight intensity in the Imidacloprid & Beta-Cyfluthrin FS 500 and in the control treatment group

In the Imidacloprid & Beta-Cyfluthrin FS 500 treatment group the flight intensity reached a maximum on evaluation day DAE 9 with an average of 5.8 bees/m²/min. On DAE 2 the maximum flight intensity in the control group was recorded with an average of 3.6 bees/m²/min.
On evaluation days DAE 11, 12, 13 and 14 the flight intensity was very low in both treatment groups, since the *Brassica napus* ceased flowering (BBCH code 67-69, end of flowering, all tubiform blossoms had bloom, in the furthest and middle third of the disc fruit settings are

visible, ligulate blossoms are dried and drop off – 71, seeds in the edge of the disc are grey).

During the entire observation period the average flight intensity was 3.3 bees/m²/min in the test substance treatment group compared to 2.3 bees/m²/min in the control treatment group.

8.3 Bee Brood

During the observation period changes and fluctuations in the relative area containing the different pre-imaginal stages, i.e. egg stage, larval and pupal stage, occurred in almost every colony of the test substance group and control group (see Table 6 to 7). The strength of the colonies was nearly on the same level in almost every hive of the test substance and the control group from the first to the second brood assessment. At the last brood assessment a decline in brood was noticed in almost every hive of the test substance and the control group. This might be caused by the relocation of the hives (22/05/2000) from a source with high nectar flow and high pollen supply to an area where the nectar flow was decreased. The continued presence of eggs showed that the queens were in good condition in all colonies of the test substance treatment group and the control group.

Table 6: Average brood development of the control treatment group from the first (DAE 5) until the fourth (DAE 47) brood assessment (hive 15, 90, 76, 114, 86 and 72)

	Assessment of combs / DAE	5	11	18	47
hive 15	No. of combs covered with bees (avg.)	10.7	10.7	10.7	11.0
	State of comb area containing eggs (avg.)*	3.0	2.0	0.0	2.0
	State of comb area containing larvae (avg.)*	2.0	2.0	1.0	2.0
	State of comb area containing pupae (avg.)*	3.0	3.0	2.5	2.5
	State of comb area containing food (avg.)*	2.7	2.7	3.0	2.3
hive 90	No. of combs covered with bees (avg.)	11.0	11.0	11.0	11.0
	State of comb area containing eggs (avg.)*	2.5	2.0	2.5	2.0
	State of comb area containing larvae (avg.)*	2.0	2.0	2.0	2.0
	State of comb area containing pupae (avg.)*	3.0	3.0	3.0	2.5
	State of comb area containing food (avg.)*	2.3	2.7	3.0	2.3
hive 76	No. of combs covered with bees (avg.)	11.0	11.0	11.0	11.0
	State of comb area containing eggs (avg.)*	2.0	0.0	0.0	2.5
	State of comb area containing larvae (avg.)*	2.0	2.0	0.0	2.5
	State of comb area containing pupae (avg.)*	3.0	3.0	2.0	2.0
	State of comb area containing food (avg.)*	3.0	2.7	2.7	2.3
hive 114	No. of combs covered with bees (avg.)	10.3	11.0	11.0	11.0
	State of comb area containing eggs (avg.)*	2.5	2.0	2.0	2.0
	State of comb area containing larvae (avg.)*	2.0	2.0	2.0	2.0
	State of comb area containing pupae (avg.)*	2.5	3.0	3.0	2.0
	State of comb area containing food (avg.)*	3.0	2.7	2.7	2.3
hive 86	No. of combs covered with bees (avg.)	10.0	10.7	10.7	11.0
	State of comb area containing eggs (avg.)*	2.0	2.0	2.0	2.0
	State of comb area containing larvae (avg.)*	2.5	2.0	2.5	2.0
	State of comb area containing pupae (avg.)*	2.5	2.5	2.5	2.0
	State of comb area containing food (avg.)*	2.0	2.7	2.7	2.7
hive 72	No. of combs covered with bees (avg.)	9.7	10.3	11.0	11.0
	State of comb area containing eggs (avg.)*	2.5	1.0	2.0	2.5
	State of comb area containing larvae (avg.)*	2.0	2.0	2.0	2.0
	State of comb area containing pupae (avg.)*	2.5	3.0	2.0	2.5
	State of comb area containing food (avg.)*	3.0	2.7	2.3	2.3

*1 = small (means below expected development status), 2 = medium (means expected development status), 3 = good (means better than expected development status), 4 = very good (means much better than expected development status)

Table 7: Average brood development of the test item treatment group from the first (DAE 5) until the fourth (DAE 47) brood assessment (hive 35, 124, 19, 108, 69 and 126)

	Assessment of combs	DAE	5	11	18	47
hive 35	No. of combs covered with bees (avg.)		10.7	10.7	11.0	10.0
	State of comb area containing eggs (avg.)*		2.0	2.5	2.0	2.5
	State of comb area containing larvae (avg.)*		2.0	2.5	2.5	2.5
	State of comb area containing pupae (avg.)*		2.5	2.5	2.5	3.0
	State of comb area containing food (avg.)*		1.7	2.7	2.7	2.0
hive 124	No. of combs covered with bees (avg.)		10.0	11.0	10.7	10.7
	State of comb area containing eggs (avg.)*		2.0	1.5	2.5	3.0
	State of comb area containing larvae (avg.)*		2.0	2.0	2.5	3.0
	State of comb area containing pupae (avg.)*		2.5	3.0	2.0	3.0
	State of comb area containing food (avg.)*		2.7	3.0	2.7	2.0
hive 19	No. of combs covered with bees (avg.)		10.3	11.0	11.0	11.0
	State of comb area containing eggs (avg.)*		2.0	2.0	2.0	2.5
	State of comb area containing larvae (avg.)*		2.0	2.0	2.0	2.5
	State of comb area containing pupae (avg.)*		3.0	3.0	2.5	3.0
	State of comb area containing food (avg.)*		2.7	2.7	2.7	2.3
hive 108	No. of combs covered with bees (avg.)		10.3	11.0	11.0	11.0
	State of comb area containing eggs (avg.)*		2.0	3.0	2.5	2.5
	State of comb area containing larvae (avg.)*		2.0	3.0	2.5	2.0
	State of comb area containing pupae (avg.)*		3.0	2.5	3.0	3.0
	State of comb area containing food (avg.)*		2.0	2.3	2.3	2.3
hive 69	No. of combs covered with bees (avg.)		10.3	10.0	10.0	11.0
	State of comb area containing eggs (avg.)*		2.5	3.0	2.0	2.5
	State of comb area containing larvae (avg.)*		1.5	2.5	3.0	2.0
	State of comb area containing pupae (avg.)*		2.5	2.5	2.5	2.5
	State of comb area containing food (avg.)*		2.0	2.7	2.7	2.7
hive 126	No. of combs covered with bees (avg.)		9.3	10.7	11.0	10.7
	State of comb area containing eggs (avg.)*		2.0	3.0	2.5	2.5
	State of comb area containing larvae (avg.)*		2.0	2.5	2.5	2.5
	State of comb area containing pupae (avg.)*		2.5	2.5	2.5	2.0
	State of comb area containing food (avg.)*		2.3	2.7	2.7	2.7

*1 = small (means below expected development status), 2 = medium (means expected development status), 3 = good (means better than expected development status), 4 = very good (means much better than expected development status)

8.4 Weight of Test Colonies

The weight of control and treatment colonies used was assessed continuously throughout the study. The records are presented in Figure 3 and 4 and in Table 8.

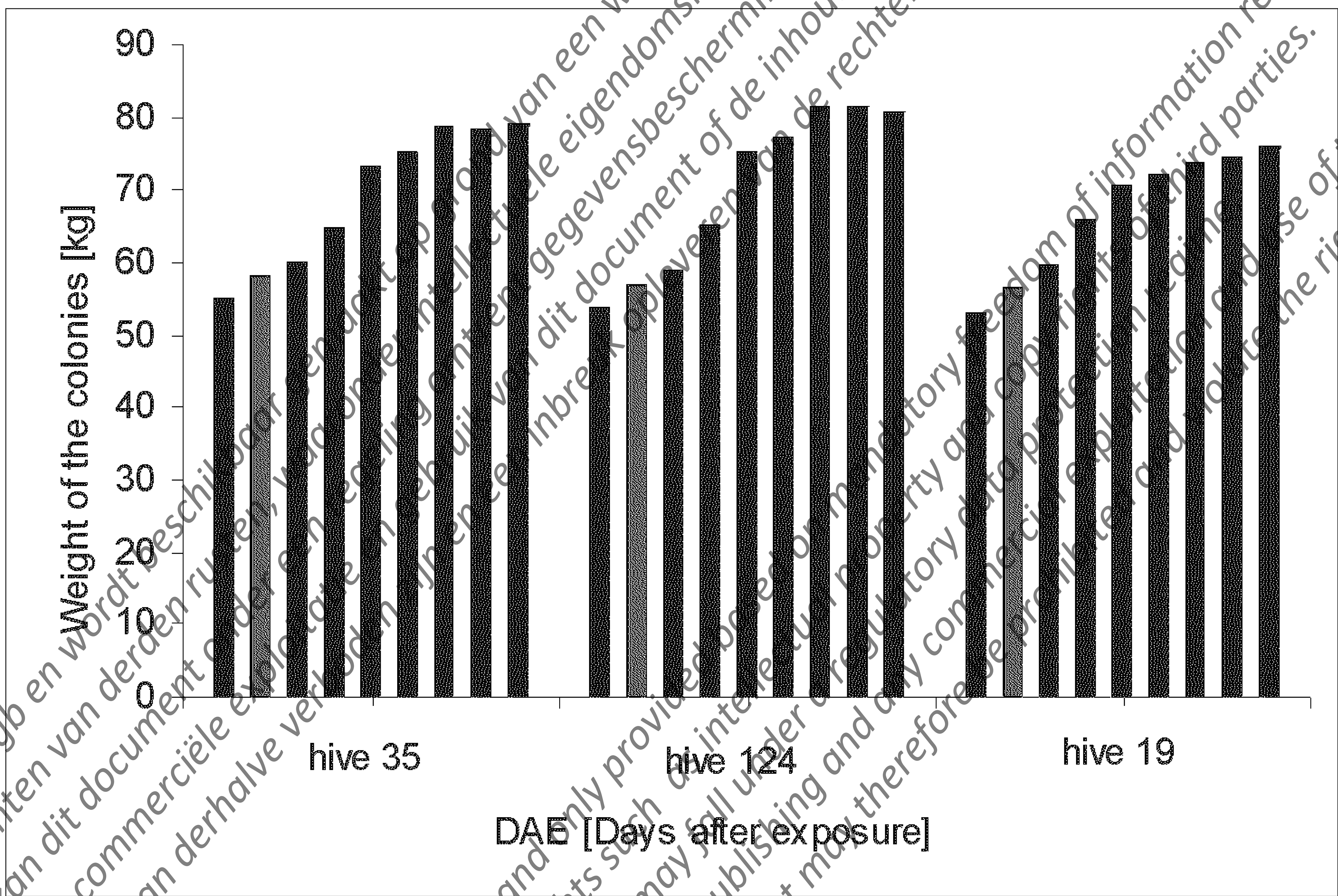


Figure 3: Weight development of the Imidacloprid & Beta-Cyfluthrin FS 500 treatment colonies

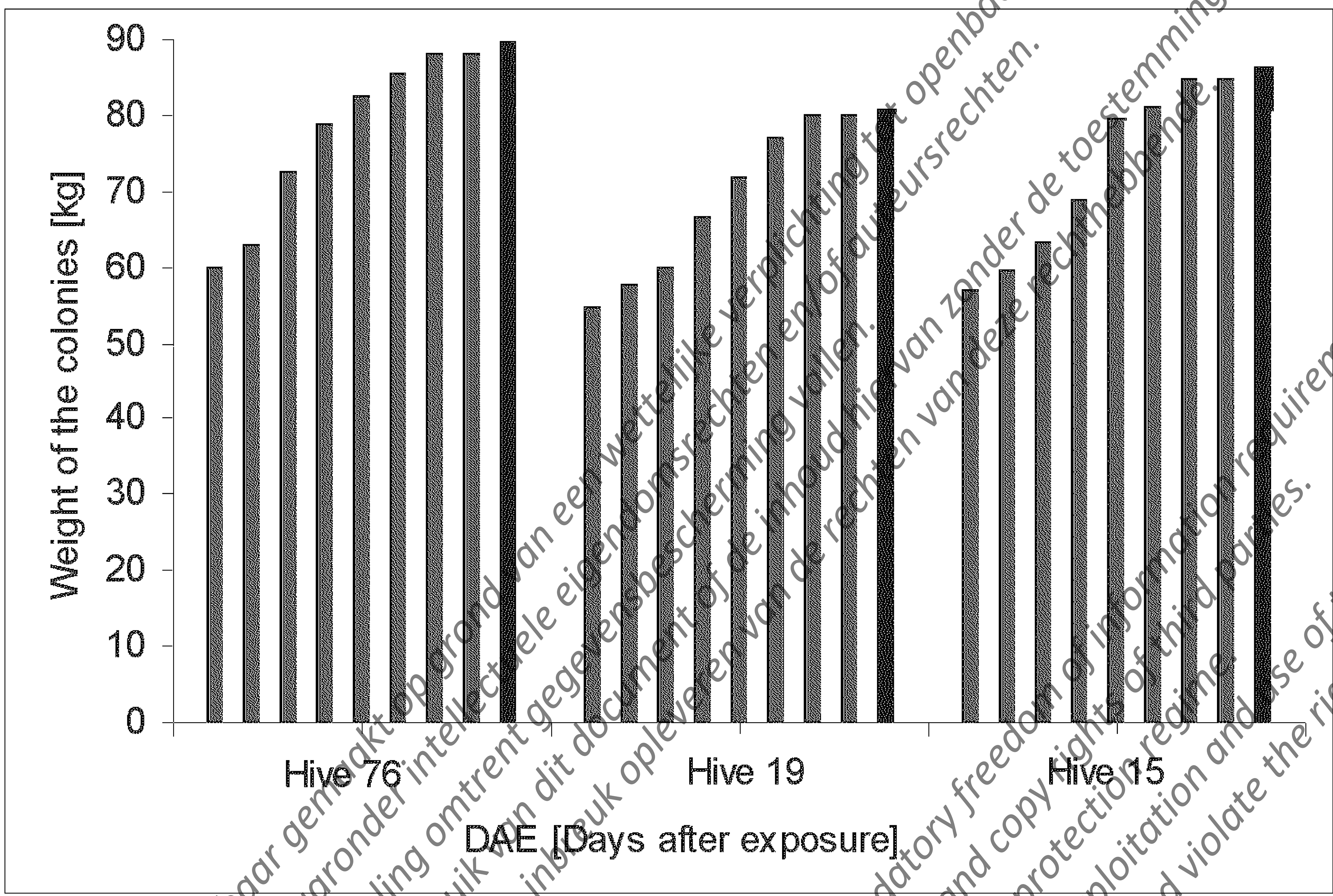


Figure 4: Weight development of the control treatment colonies

Table 8: Weight development of the three colonies of the 1st group from the treatment and the control

Date	DAE	Weights of the three remaining colonies of the 1st group [kg]					
		Imidacloprid & Beta-Cyfluthrin FS 500 Colonies			Control Colonies		
		Hive No. 35	Hive No. 124	Hive No. 19	Hive No. 76	Hive No. 90	Hive No. 15
27.04.2000	0	54.5	52.8	51.3	58.0	53.7	55.7
28.04.2000	1	55.1	53.8	53.1	59.9	54.8	57.0
29.04.2000	2	57.6	55.8	56.1	62.0	56.7	58.7
30.04.2000	3	57.9	56.7	56.6	63.0	57.5	59.5
01.05.2000	4	58.3	57.5	57.4	64.1	58.4	60.5
02.05.2000	5	-	-	- 0.59 g[1)]	+ 5.0[3)]	-	-
02.05.2000	5	59.9	59.0	59.8	72.7	60.0	63.4
03.05.2000	6	62.0	61.6	62.5	75.6	62.8	65.6
04.05.2000	7	64.7	65.2	65.8	78.9	66.6	68.9
05.05.2000	8	+ 4.95[2)]	+ 4.95[2)]	-	-	-	+ 5.0[4)]
05.05.2000	8	71.6	73.0	68.4	80.2	69.3	76.8
06.05.2000	9	73.2	75.2	70.4	82.4	71.9	79.6
07.05.2000	10	74.5	76.8	71.8	85.2	75.2	79.3
08.05.2000	11	-	-	-	-	-	- 1.35[5)]
08.05.2000	11	75.3	77.2	72.1	85.6	76.8	81.1
09.05.2000	12	77.2	79.6	72.5	87.2	78.9	83.4
10.05.2000	13	78.6	81.6	73.8	88.1	80.1	84.7
11.05.2000	14	79.0	81.9	74.8	88.6	80.4	85.1
12.05.2000	15	78.5	81.3	74.3	88.3	80.0	84.9
13.05.2000	16	78.6	81.4	75.1	89.0	80.4	85.6
14.05.2000	17	79.0	80.6	75.8	89.5	80.8	86.2
15.05.2000	18	78.8	80.5	75.7	89.7	80.0	80.5
Weight development in kg		+ 24.3	+ 27.7	+ 24.4	+ 31.7	+ 26.3	+ 24.8
Weight development in %		+ 44.6 %	+ 52.5	+ 47.6	+ 54.7	+ 49.0	+ 44.5

1): one full comb was replaced by an empty comb (-0.586 kg)
2) one body with empty combs was set-up (+ 4.95 kg)
3) and 4): One body with empty combs was set-up (+ 5.0 kg)
5): one brood comb was replaced by an empty comb (-1.35 kg)

In the Imidacloprid & Beta-Cyfluthrin FS 500 treatment group the weights of all three hives increased during the 27/04/2000 - 11/05/2000 observation period. After this time no noticeable change in the weight of the colonies was recorded until the 15/05/2000. In the control colonies the weights of all three hives increased during the 27/04/2000 - 11/05/2000 observation period similar to the weight of the colonies of the Imidacloprid & Beta-Cyfluthrin FS 500 treatment group. After the 11/05/2000 no noticeable increase or decrease of the colony weight in control and treatment was measured.

8.5 Behaviour of the Bees

The bees visiting the oil-seed rape plants originating from seeds dressed with Imidacloprid & Beta-Cyfluthrin FS 500 showed normal and intensive pollen and nectar collection behaviour with no differences to the control treatment group. On all evaluation days no abnormal behaviour of the bees on the crop and around the colonies was observed compared to the control.

8.6 Analytical Findings

Table 9 shows the results of the analytical research carried out with nectar, pollen and honey.

Table 9: Analytical findings of the samples taken during the study

Test substance	Imidacloprid & Beta-Cyfluthrin FS 500					
Test organism	*Apis mellifera*					
Exposure	Oil-seed rape					
Sample material	Control field			Test substance field		
Analysed for [mg/kg]	Hydroxy-Imida-cloprid	Olefin-Imida-cloprid	Imida-cloprid	Hydroxy-Imida-cloprid	Olefin-Imida-cloprid	Imida-cloprid
Nectar from the comb	n.d.	n.d.	n.d.	n.d.	n.d.	< LOQ
Pollen from the comb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nectar from the comb	n.d.	n.d.	n.d.	n.d.	n.d.	< LOQ
Pollen from the comb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nectar from the comb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Honey from the comb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nectar from the blossoms	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Limit of quantitation: 0.005 mg/kg for Imidacloprid and Hydroxy-Metabolite, 0.01 mg/kg for the Olefin-Metabolite
< 0.005 and < 0.010 = Residues below the limit of quantitation (< LOQ)
Limit of detection: 0.0015 mg/kg for Imidacloprid and Hydroxy-Metabolite, 0.003 mg/kg for the Olefin-Metabolite
n.d.: Residues below the limit of detection

The results indicated, that in 40 out of 42 samples the residues of the test substance or the metabolites were below the limit of detection. In two out of 42 samples the residues were below the limit of quantitation.

9 Discussion and Conclusions

There were no adverse effects of the Imidacloprid & Beta-Cyfluthrin FS 500 treatment on foraging activities of the bees, colony weight and development or mortality. No behavioural impacts (e.g. apathy, exaggerated motility, discoordinated movements) were observed on the honey bees collecting rape nectar and pollen in comparison to the bees from the control field.

Likewise, in the analytical part of this study no residues of metabolites of the test substance were found in pollen, nectar or honey. In the nectar collected out of the combs residues of the test substance below the limit of quantitation (< 0.005 mg/kg) were found. In the other samples (pollen and honey from the combs and nectar from the blossoms) no residues of the test substance were found.

10 Archiving

This study was allocated the study code 99398/01-BFEU. The final report was prepared in two original signed copies. For the periods demanded by the principles of GLP (and at least 10 years) the following documents and materials will be archived:

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

All documents and materials will be stored in the archives of 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.

11 References

Deutsches Institut für Normung in Berlin (DIN 10753), August 1994

Deutsches Institut für Normung in Berlin (DIN 10758), May 1997

EPPO (1992): Guideline on test methods for evaluating the side-effects of plant protection products on honey bees.- EPPO Bulletin **22**, 203 - 208.

SETAC (1995): Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides. M. R. Lynch (Ed.), 49-51

12 Distribution

12.1 Study Plan

Original: Sponsor (1 x)

Testing facility (1 x)

12.2 Final Report

Original: Sponsor (1 x)

Testing facility (1 x)

12.3 Raw Data

Original: Testing facility

Copy Sponsor (1 x)

13 Appendix

Appendix 1

Table 10: Weather conditions during the trial

Date	DAE	Temperature min / max [°C]	% rel. humidity min/max	Precipitation [mm]	Cloud formation at time of evaluation [%]
28.04.2000	1	10.5 / 23.0	50 / 85	-	60
29.04.2000	2	10.0 / 24.0	46 / 88	-	50
30.04.2000	3	12.0 / 25.0	46 / 85	1	100
01.05.2000	4	12.0 / 18.0	68 / 85	0.3	100
02.05.2000	5	11.5 / 22.0	47 / 87	0.8	2
03.05.2000	6	7.0 / 22.0	45 / 86	-	0
04.05.2000	7	7.0 / 22.0	35 / 84	-	10
05.05.2000	8	6.0 / 23.5	33 / 87	-	0
06.05.2000	9	6.0 / 25.0	31 / 80	-	0
07.05.2000	10	7.5 / 25.0	33 / 85	-	0
08.05.2000	11	9.0 / 25.5	33 / 80	-	0
09.05.2000	12	8.5 / 26.0	30 / 85	-	30
10.05.2000	13	6.5 / 29.0	28 / 86	-	0
11.05.2000	14	5.5 / 29.0	26 / 88	-	0

DAE Days after begin of exposure

Appendix 2

Table 11: Individual results of the evaluations of mortality (numbers of dead bees) in the Imidacloprid & Beta-Cyfluthrin FS 500 treatment group (first colony group)

		Mortality (number of dead bees)					
		Linen sheets in the field	Linen sheet in front of the hives	Colony 1 Hive 19	Colony 2 Hive 124	Colony 3 Hive 35	Ø/Colony and Day
Date	DAE		LS	BT	BT	BT	BT+LS
28.04.2000	1	0	52	1	4	2	19.7
29.04.2000	2	0	24	0	3	1	9.3
30.04.2000	3	0	12	0	1	4	5.7
01.05.2000	4	0	18	5	2	0	8.3
02.05.2000	5	0	25	0	0	0	8.3
03.05.2000	6	0	63	2	13	2	26.7
04.05.2000	7	5	12	4	7	3	8.7
05.05.2000	8	0	1	3	4	3	3.7
06.05.2000	9	0	10	2	1	1	4.7
07.05.2000	10	1	17	0	0	3	6.7
08.05.2000	11	1	5	3	2	2	4.0
09.05.2000	12	3	16	0	0	0	5.3
10.05.2000	13	0	6	0	0	0	2.0
11.05.2000	14	1	9	0	1	1	3.7
Mean		0.8	19.3	1.4	2.7	1.6	8.3
STD		1.5	17.7	1.7	3.5	1.4	6.9

DAE = Days after begin of exposure
BT+LS = Bee traps and linen sheet in front of the hives
STD = Standard deviation

Appendix 2 (continued)

Table 12: Individual results of the evaluations of mortality (numbers of dead bees) in the control treatment group (first colony group)

		Mortality (number of dead bees)					
Date	DAE	Linen sheets in the field	Linen sheet in front of the hives LS	Colony 1 Hive 76 BT	Colony 2 Hive 90 BT	Colony 3 Hive 15 BT	∅/Colony and Day BT+LS
28.04.2000	1	0	37	0	1	2	13.3
29.04.2000	2	0	23	0	0	0	7.7
30.04.2000	3	0	21	2	0	0	7.7
01.05.2000	4	0	17	0	4	0	7.0
02.05.2000	5	0	27	0	2	1	10.0
03.05.2000	6	1	91	1	0	1	31.0
04.05.2000	7	0	29	2	2	1	11.3
05.05.2000	8	0	17	2	0	4	7.7
06.05.2000	9	0	39	0	0	0	13.0
07.05.2000	10	1	20	0	1	0	7.0
08.05.2000	11	0	16	2	0	0	6.0
09.05.2000	12	0	75	0	0	0	25.0
10.05.2000	13	0	22	0	0	0	7.3
11.05.2000	14	0	42	0	0	0	14.0
Mean		0.1	34.0	0.6	0.7	0.5	12.0
STD		0.4	23.5	0.9	1.2	1.1	7.7

DAE = Days after begin of exposure
BT+LS = Bee traps and linen sheet in front of the hives
STD = Standard deviation

Appendix 3

Table 13: Average flight intensity (number of bees per m² *Brassica napus*/assessment/min) in the Imidacloprid & Beta-Cyfluthrin FS 500 treatment group

Date	DAE	Area* 1	Area* 2	Area* 3	Area* 4	Area* 5	Ø/m²/min	STD	% cloud cover
28.04.2000	1	5.0	4.0	2.0	3.0	12.0	5.2	4.0	60
29.04.2000	2	6.0	6.0	1.0	5.0	7.0	5.0	2.3	50
30.04.2000	3	1.0	1.0	1.0	2.0	2.0	1.4	0.5	100
01.05.2000	4	0.0	0.0	0.0	1.0	1.0	0.4	0.5	100
02.05.2000	5	3.0	4.0	5.0	4.0	5.0	4.2	0.8	2
03.05.2000*	6	2.8	3.3	4.5	5.0	6.0	4.3	1.3	0
04.05.2000*	7	5.2	4.0	6.0	4.5	6.8	5.3	1.1	16
05.05.2000*	8	6.0	4.8	6.3	3.3	7.0	5.5	1.5	0
06.05.2000	9	6.0	6.0	5.0	5.0	7.0	5.8	0.8	0
07.05.2000	10	1.0	0.0	2.0	5.0	8.0	3.2	3.3	0
08.05.2000*	11	2.8	2.0	1.8	2.5	3.3	2.5	0.6	50
09.05.2000	12	2.0	1.0	0.0	0.0	4.0	1.4	1.7	30
10.05.2000	13	0.0	3.0	1.0	0.0	3.0	1.4	1.5	0
11.05.2000	14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
Mean		2.9	2.8	2.5	2.9	5.2	3.3	1.4	29
STD		2.4	2.1	2.3	2.0	3.3	2.0	1.1	37.1

DAE Days after beginning of exposure
STD Standard deviation
*Distribution of the plots is given in Appendix 7, Figure 7
Ø of 4 assessments/area/day

Appendix 3 (continued)

Table 14: Average flight intensity (number of bees per m² *Brassica napus*/assessment/min) in the control treatment group

Date	DAE	Area* 1	Area* 2	Area* 3	Area* 4	Area* 5	Ø/m²/min	STD	% cloud cover
28.04.2000	1	4.0	3.0	5.0	0.0	4.0	3.2	1.9	60
29.04.2000	2	4.0	6.0	4.0	3.0	1.0	3.6	1.8	50
30.04.2000	3	1.0	3.0	1.0	0.0	0.0	1.0	1.2	100
01.05.2000	4	0.0	1.0	1.0	1.0	0.0	0.6	0.5	100
02.05.2000	5	2.0	3.0	1.0	2.0	3.0	2.2	0.8	2
03.05.2000	6	3.0	3.8	3.3	3.0	3.5	3.3	0.3	0
04.05.2000	7	2.0	4.3	4.3	2.0	4.0	3.3	1.2	38
05.05.2000	8	1.8	1.5	2.8	2.5	2.5	2.2	0.5	0
06.05.2000	9	1.0	6.0	4.0	1.0	2.0	2.8	2.2	0
07.05.2000	10	1.0	4.0	4.0	0.0	2.0	2.2	1.8	0
08.05.2000	11	1.0	1.5	2.8	1.8	1.5	1.7	0.7	40
09.05.2000	12	2.0	2.0	5.0	1.0	3.0	2.6	1.5	30
10.05.2000	13	2.0	0.0	1.0	1.0	2.0	1.2	0.8	0
11.05.2000	14	2.0	2.0	2.0	3.0	4.0	2.6	0.9	0
Mean		1.9	2.9	2.9	1.5	2.3	2.3	1.2	30
STD		1.1	1.8	1.5	1.1	1.4	0.9	0.6	36.6

DAE Days after beginning of exposure
STD Standard deviation
*Distribution of the plots is given in Appendix 7, Figure 9
Ø of 4 assessments/area/day

Appendix 4

Table 15: Assessment of bee colony development of the Imidacloprid & Beta-Cyfluthrin FS 500 treatment group (first colony group)

	Colony1 (19)	Colony 2 (124)	Colony 3 (35)
1st assessment after begin of exposure: 02.05.2000 (DAE 5), body 1/2/3			
Strength (No. of combs covered with bees)**	10/11/10	10/11/9	11/11/10
No. of brood combs	7/9	4/7	5/8
Status of comb area containing food*	3/3/2	3/3/2	1/2/2
Status of comb area containing pollen*	3/3/0	3/3/0	1/1/0
Status of comb area containing eggs*	2/2	2/2	2/2
Status of comb area containing larvae*	2/2	2/2	2/2
Status of comb area containing pupae*	3/3	2/3	2/3
2nd assessment after begin of exposure: 08.05.2000 (DAE 11), body 1/2/3			
Strength (No. of combs covered with bees)**	11/11/11	11/11/11	10/11/11
No. of brood combs	7/8	5/9	6/9
Status of comb area containing food*	2/3/3	3/3/3	2/3/3
Status of comb area containing pollen*	3/3/0	3/3/0	3/3/0
Status of comb area containing eggs*	2/2	1/2	2/3
Status of comb area containing larvae*	2/2	2/2	3/2
Status of comb area containing pupae*	3/3	3/3	2/3
3rd assessment after begin of exposure: 15.05.2000 (DAE 18), body 1/2/3			
Strength (No. of combs covered with bees)**	11/11/11	10/11/11	11/11/11
No. of brood combs	6/6	6/8	6/7
Status of comb area containing food*	2/3/3	2/3/3	2/3/3
Status of comb area containing pollen*	2/2/0	3/3/0	3/3/0
Status of comb area containing eggs*	2/2	2/3	2/2
Status of comb area containing larvae*	2/2	2/3	2/3
Status of comb area containing pupae*	2/3	2/2	3/2
4th assessment after begin of exposure: 13.06.2000 (DAE 47), body 1/2/3			
Strength (No. of combs covered with bees)**	11/11/11	11/11/10	10/10/10
No. of brood combs	5/7	3/8	7/9
Status of comb area containing food*	2/3/2	2/2/2	2/2/2
Status of comb area containing pollen*	2/2/0	3/3/0	2/2/0
Status of comb area containing eggs*	2/3	3/3	3/2
Status of comb area containing larvae*	2/3	3/3	2/3
Status of comb area containing pupae*	3/3	2/2	3/3

*Assessment: 1 = small (means below expected development status), 2 = medium (means expected development status), 3 = good (means better than expected development status), 4 = very good (means much better than expected development status)

**The colonies were comprised of one food and two brood bodies

Appendix 4 (continued)

Table 16: Assessment of bee colony development of the Imidacloprid & Beta-Cyfluthrin FS 500 treatment group (second colony group)

	Colony1 (126)	Colony 2 (69)	Colony 3 (108)
1st assessment after begin of exposure: 02.05.2000 (DAE 5), body 1/2/3			
Strength (No. of combs covered with bees)**	9/10/9	10/11/10	10/11/10
No. of brood combs	5/9	4/9	9/10
Status of comb area containing food*	2/3/2	2/2/2	2/2/2
Status of comb area containing pollen*	3/3/0	3/3/0	3/3/0
Status of comb area containing eggs*	2/2	2/3	2/2
Status of comb area containing larvae*	2/2	1/2	2/2
Status of comb area containing pupae*	3/2	2/3	3/3
2nd assessment after begin of exposure: 08.05.2000 (DAE 11), body 1/2/3			
Strength (No. of combs covered with bees)**	11/11/10	10/10/10	11/11/11
No. of brood combs	6/8	4/10	9/10
Status of comb area containing food*	2/3/3	2/3/3	2/2/3
Status of comb area containing pollen*	3/3/0	2/2/0	3/3/0
Status of comb area containing eggs*	3/3	3/3	3/3
Status of comb area containing larvae*	2/3	2/3	3/3
Status of comb area containing pupae*	3/2	2/3	2/3
3rd assessment after begin of exposure: 15.05.2000 (DAE 18), body 1/2/3			
Strength (No. of combs covered with bees)**	11/11/11	10/10/10	11/11/11
No. of brood combs	7/8	4/10	8/9
Status of comb area containing food*	2/3/3	2/3/3	2/2/3
Status of comb area containing pollen*	3/3/0	2/3/0	3/3/0
Status of comb area containing eggs*	3/2	2/2	3/2
Status of comb area containing larvae*	2/3	3/3	2/3
Status of comb area containing pupae*	2/3	2/3	3/3
4th assessment after begin of exposure: 13.06.2000 (DAE 47), body 1/2/3			
Strength (No. of combs covered with bees)**	11/10/11	11/11/11	11/11/11
No. of brood combs	8/8	5/7	8/10
Status of comb area containing food*	2/3/3	2/3/3	2/2/3
Status of comb area containing pollen*	3/2/0	2/2/0	2/2/0
Status of comb area containing eggs*	2/3	3/2	3/2
Status of comb area containing larvae*	3/2	2/2	2/2
Status of comb area containing pupae*	2/2	2/3	3/3

*Assessment: 1 = small (means below expected development status), 2 = medium (means expected development status), 3 = good (means better than expected development status), 4 = very good (means much better than expected development status)

**The colonies were comprised of one food and two brood bodies

Appendix 4 (continued)

Table 17: Assessment of bee colony development of the control treatment group (first colony group)

	Colony1 (15)	Colony 2 (90)	Colony 3 (76)
1st assessment after begin of exposure: 02.05.2000 (DAE 5), body 1/2/3			
Strength (No. of combs covered with bees)**	11/11/10	11/11/11	11/11/11
No. of brood combs	6/8	9/8	6/8
Status of comb area containing food*	3/3/2	2/3/2	3/3/3
Status of comb area containing pollen*	3/3/0	3/2/0	3/3/0
Status of comb area containing eggs*	3/3	3/2	2/2
Status of comb area containing larvae*	2/2	2/2	2/2
Status of comb area containing pupae*	3/3	3/3	3/3
2nd assessment after begin of exposure: 08.05.2000 (DAE 11), body 1/2/3			
Strength (No. of combs covered with bees)**	10/11/11	11/11/11	11/11/11
No. of brood combs	6/8	8/8	6/9
Status of comb area containing food*	2/3/3	2/3/3	2/3/3
Status of comb area containing pollen*	3/3/0	3/3/0	3/3/0
Status of comb area containing eggs*	2/2	2/2	0/0
Status of comb area containing larvae*	2/2	2/2	2/2
Status of comb area containing pupae*	3/3	3/3	3/3
3rd assessment after begin of exposure: 15.05.2000 (DAE 18), body 1/2/3			
Strength (No. of combs covered with bees)**	10/11/11	11/11/11	11/11/11
No. of brood combs	6/3	7/6	6/4
Status of comb area containing food*	3/3/3	3/3/3	2/3/3
Status of comb area containing pollen*	3/3/0	3/3/0	3/2/0
Status of comb area containing eggs*	0/0	3/2	0/0
Status of comb area containing larvae*	2/0	2/2	0/0
Status of comb area containing pupae*	3/2	3/3	2/2
4th assessment after begin of exposure: 13.06.2000 (DAE 47), body 1/2/3			
Strength (No. of combs covered with bees)**	11/11/11	11/11/11	11/11/11
No. of brood combs	5/8	6/8	5/5
Status of comb area containing food*	2/2/3	2/2/3	2/2/3
Status of comb area containing pollen*	2/2/0	2/2/0	3/2/0
Status of comb area containing eggs*	2/2	2/2	2/3
Status of comb area containing larvae*	2/2	2/2	2/3
Status of comb area containing pupae*	2/3	2/3	2/2

*Assessment: 1 = small (means below expected development status), 2 = medium (means expected development status), 3 = good (means better than expected development status), 4 = very good (means much better than expected development status)

**The colonies were comprised of one food and two brood bodies

Appendix 4 (continued)

Table 18: Assessment of bee colony development of the control treatment group (second colony group)

	Colony1 (114)	Colony 2 (86)	Colony 3 (72)
1st assessment after begin of exposure: 02.05.2000 (DAE 5), body 1/2/3			
Strength (No. of combs covered with bees)**	10/11/10	10/10/10	9/10/10
No. of brood combs	7/7	8/9	7/7
Status of comb area containing food*	3/3/3	3/3/0	3/3/3
Status of comb area containing pollen*	3/2/0	2/2/0	3/3/0
Status of comb area containing eggs*	3/2	2/2	2/3
Status of comb area containing larvae*	2/2	2/3	2/2
Status of comb area containing pupae*	3/2	2/3	2/3
2nd assessment after begin of exposure: 08.05.2000 (DAE 11), body 1/2/3			
Strength (No. of combs covered with bees)**	11/11/11	10/11/11	10/11/10
No. of brood combs	6/8	8/8	7/8
Status of comb area containing food*	2/3/3	2/3/3	2/3/3
Status of comb area containing pollen*	3/3/0	3/3/0	3/3/0
Status of comb area containing eggs*	2/2	2/2	0/2
Status of comb area containing larvae*	2/2	2/2	2/2
Status of comb area containing pupae*	3/3	3/2	3/3
3rd assessment after begin of exposure: 15.05.2000 (DAE 18), body 1/2/3			
Strength (No. of combs covered with bees)**	11/11/11	11/10/11	11/11/11
No. of brood combs	6/8	7/8	5/7
Status of comb area containing food*	2/3/3	2/3/3	2/2/3
Status of comb area containing pollen*	3/2/0	2/3/0	3/3/0
Status of comb area containing eggs*	2/2	2/2	2/2
Status of comb area containing larvae*	2/2	2/3	2/2
Status of comb area containing pupae*	3/3	3/2	2/2
4th assessment after begin of exposure: 13.06.2000 (DAE 47), body 1/2/3			
Strength (No. of combs covered with bees)**	11/11/11	11/11/11	11/11/11
No. of brood combs	4/6	8/4	6/8
Status of comb area containing food*	2/2/3	2/3/3	2/2/3
Status of comb area containing pollen*	2/2/0	2/1/0	3/3/0
Status of comb area containing eggs*	2/2	2/2	2/3
Status of comb area containing larvae*	2/2	2/2	2/2
Status of comb area containing pupae*	2/2	2/2	2/3

*Assessment: 1 = small (means below expected development status), 2 = medium (means expected development status), 3 = good (means better than expected development status), 4 = very good (means much better than expected development status)

**The colonies were comprised of one food and two brood bodies

Appendix 5

Table 19: Results of recording of the flowering stages

		BBCH code of the oil-seed spring rape	
Date	DAE	Control field	Test substance field
11.04.2000	-16	51	51
20.04.2000	-7	53	53 – 55
23.04.2000	-4	57	59
25.04.2000	-2	59	60
27.04.2000	0	60 – 61	61 – 62
28.04.2000	1	61 – 62	62 – 63
30.04.2000	3	64	64 – 65
02.05.2000	5	64 – 65	65
05.05.2000	8	65 – 66	65 – 66
09.05.2000	12	67	67 – 69
12.05.2000	15	69	> 69

DAE: Days after the start of exposure

Appendix 6

Figure 5: Photograph of the hives adjacent to the crop with the wooden bee traps placed over their entrances and the linen sheet in front of the hives

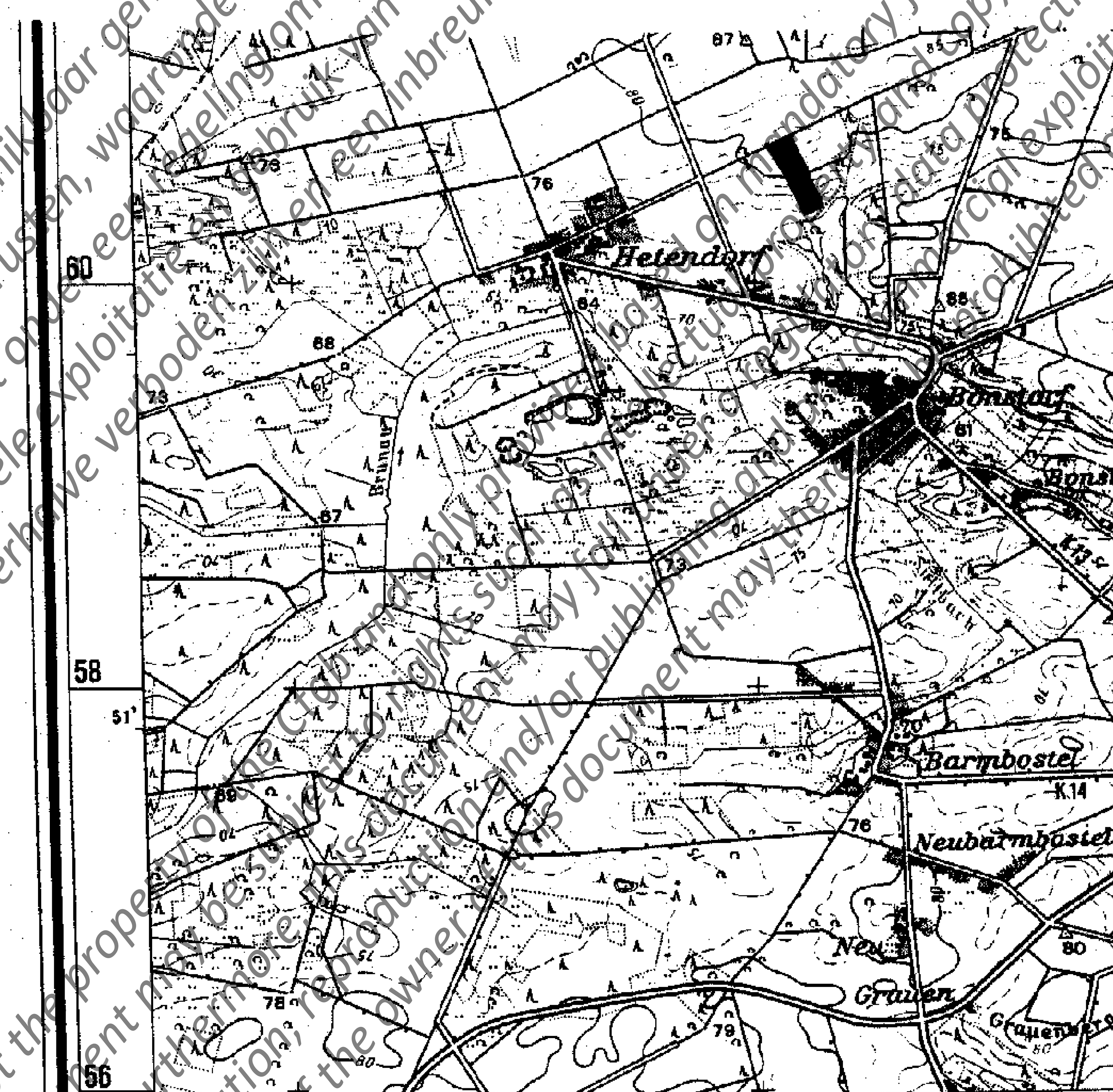
Appendix 7

Allgemeines und Landkarte

Trial code GAB Versuchscode GAB:	[redacted]	Trial number sponsor: Versuchscode Auftraggeber:	
Location: Ort:	Hetendorf	Zip code: Postleitzahl:	29320
Region: Region:	LK Celle	Country: Land:	GERMANY
Map: Landkarte:	L 3126 "Munster"		
Scale (≤ 1:50000): Maßstab	1:50000	Meters above sea-level: Höhe über dem Meer:	80

Mark the test site visible!

Geogr. Breite: Nord 52° 52,4'
Geogr. Länge: Ost 10° 2,7'



Date: 05-05-2000 [redacted]

Figure 6: General map of the area around the test substance group field (test field marked black)

Appendix 7 (continued)

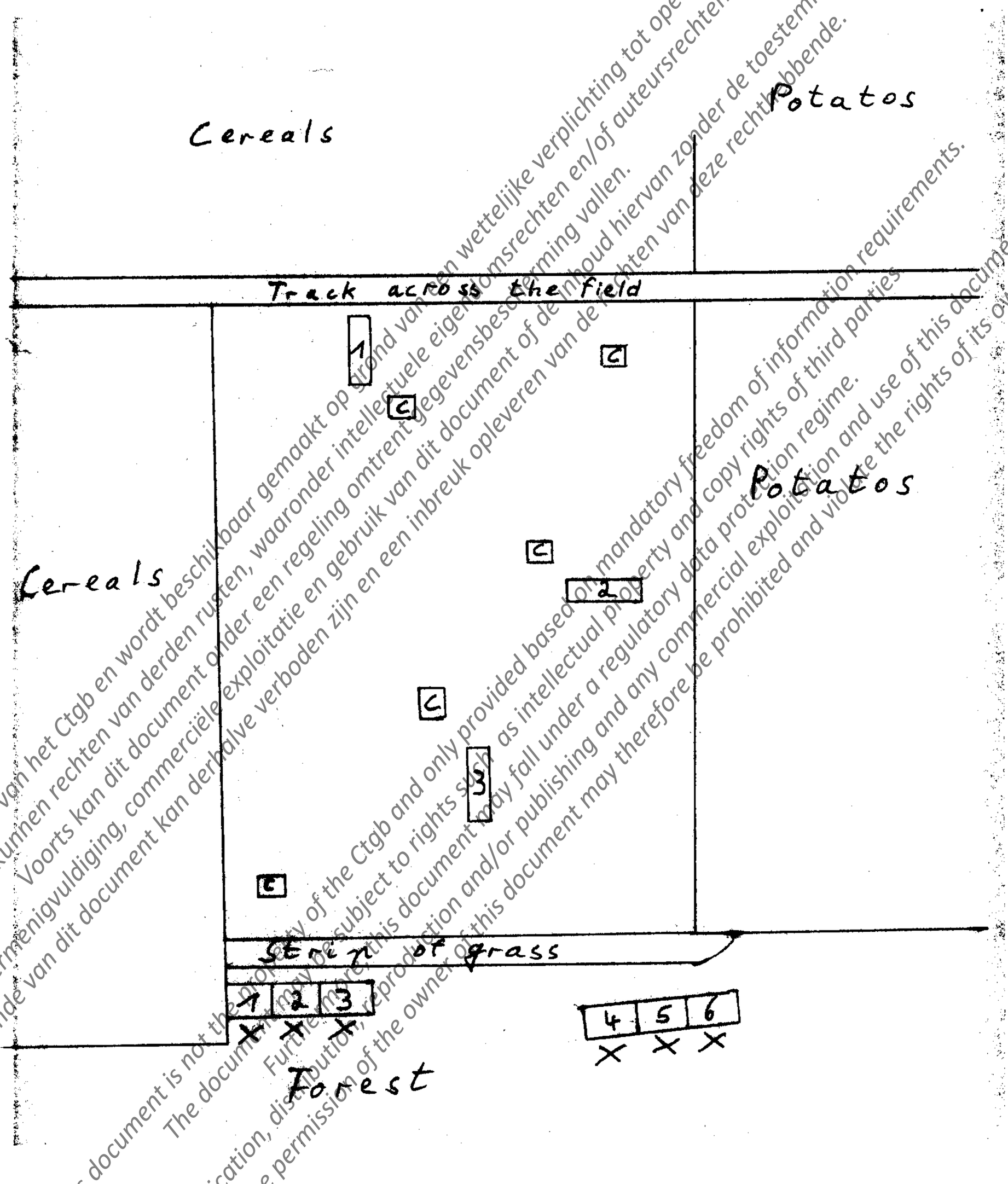


Figure 7: Map of the test substance treatment field

1 – 3: Linen sheets
c: Counting areas for flight activity assessments
1, 2, 3 (XXX): Bee hives with the dead bee traps (first colony group)
3, 4, 5 (XXX): second colony group

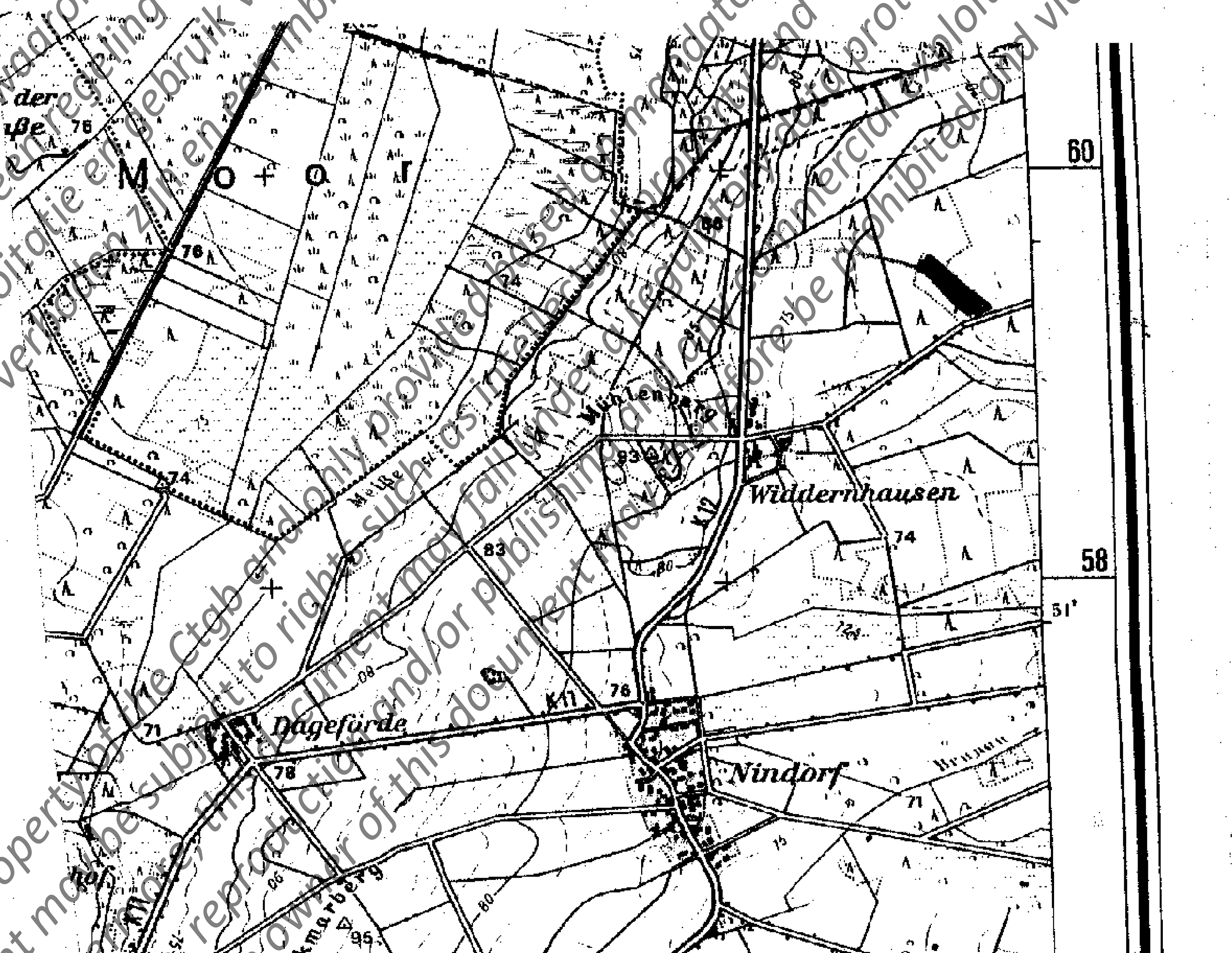
Appendix 7 (continued)

Allgemeines und Landkarte

Trial code GAB Versuchscode GAB:	[redacted]	**Trial number sponsor:** Versuchscode Auftraggeber:	
Location: Ort:	Hetendorf	**Zip code:** Postleitzahl:	29320
Region: Region:	LK Celle	**Country:** Land:	GERMANY
Map: Landkarte:	TK L 3124 "Soltau"		
Scale (≤ 1:50000): Maßstab	1:50 000	**Meters above sea-level:** Höhe über dem Meer:	~72

Mark the test site visible!

Geogr. Breite: Nord 52°51,9'
Geogr. Länge: Ost 9°59,7'



Date: 05.05.00

[redacted]

Figure 8: General map of the area around the control group field (test field marked black)

Appendix 7 (continued)

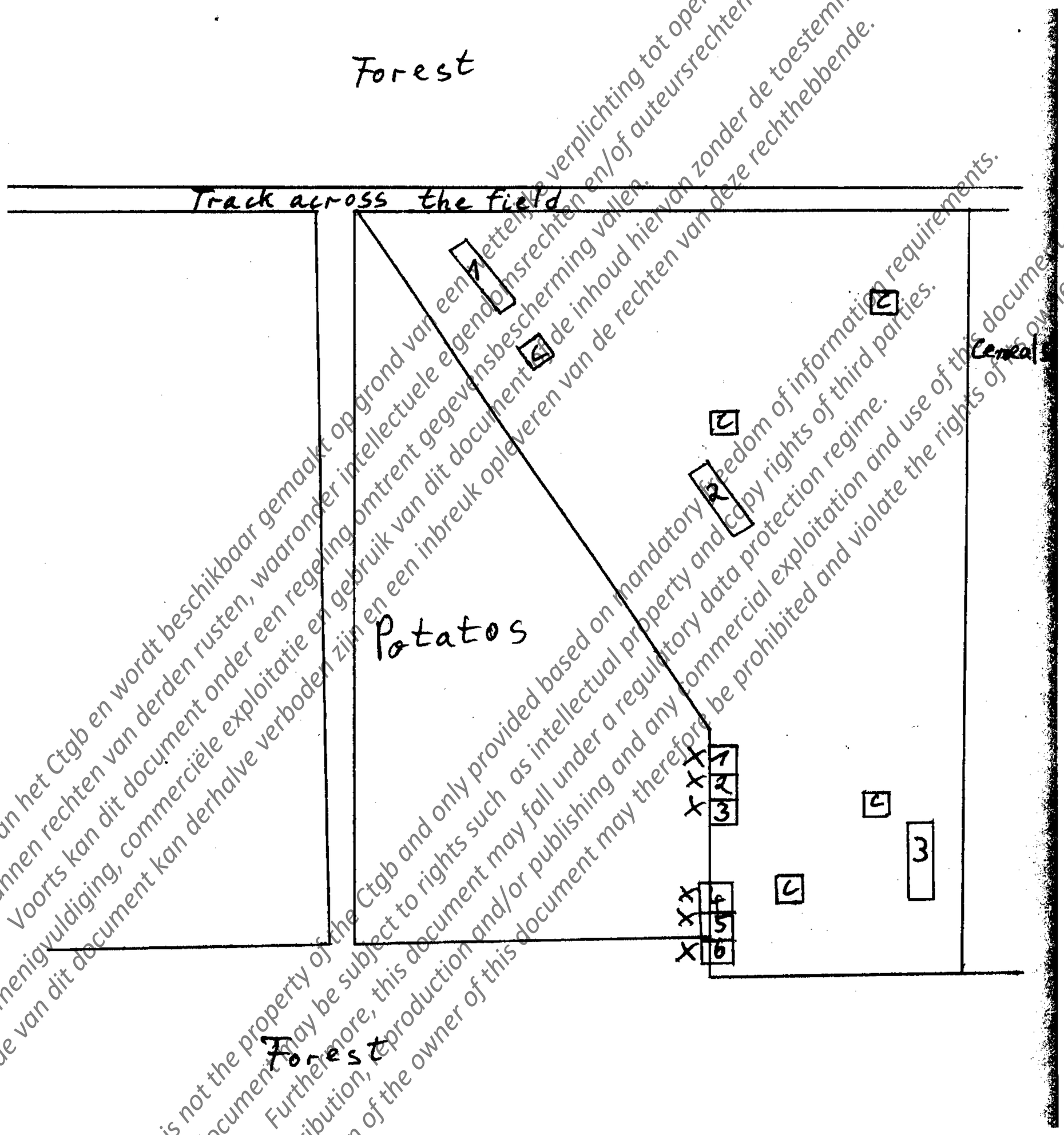


Figure 9: Map of the control field
1 – 3: Linen sheets
c: Counting areas for flight activity assessments
1, 2, 3 (XXX): Bee hives with the dead bee traps (first colony group)
3, 4, 5 (XXX): second colony group

Appendix 8 Certificate of analysis of test substance and of the analysis of the dressed rape seed

Bayer

[redacted] Monheim
Geb.: 6200

Pflanzenschutzzentrum
PF-PM/PPA

[redacted]

PF-PM Beiztechnikum
[redacted]

Geb. 5912

HWJ	Biol. Entwicklung Insektizide		KE
BR			HWS
DE	0 6. Juli 1999		DOK
KD	z. Ktns.	z.Vbl.	Uml.
	Ablage:		

Leverkusen, 02.07.99
Auftrags-Nr.: 621/99

Substanzlieferung

Aufgrund Ihrer Bestellung erhalten Sie die nachfolgend genannte Substanz.

Menge: 1,000 Liter
Artikel-/Aufbau-Nr.: 0195939
Produkt: FCR 4545 + NTN 33893 FS 500

Pt.-/Fl.-Nr.: 06200/0059/0055
TOX/FAR-NR.: 4867-00
Gehalt: s. Anlage
Frei bis: 03.09.99

Verwendung: Bienenversuch

Bemerkung: [redacted]

Mit freundlichen Grüßen

[redacted]

Anlage (n):

Versand ab : .... Datum .... Unterschrift

Eingang in : .... Datum .... Unterschrift

Bitte nach Empfang zurück an PF-PM/PPA.

Appendix 8 (continued)

Bayer AG
PF-PM/PPA

05.03.99

Approval of Preparation Sample

Preparation Sample TOX 4867

Sample: *FCR 4545 00080 FS 06200/0059*
NTN 33893 00420

Development-No.: *0195939*

Indication: *Insecticide*

Active Ingredients: *1. FCR 4545*
2. NTN 33893

Formulation No.: *0055(3.Pf)* *based on Formulation No.: 06200/0059*

Origin of sample: *PF-E/FT*

Responsible Analyst: [redacted] **Laboratory:** *FT-EA*

Analytical Methods: *1.GLC, 2.HPLC*

Approvals:

TOX	Purity		Approved until	Date of Analysis	Comment
4867-00	*1. 77.25* *2. 433.53*	*g/l* *g/l*	*03.09.99*	*03.03.99*	

[redacted]

A reserve sample will be retained.

Appendix 8 (continued)

PF-E-FT-EA

[redacted]

Bayer

Orderer: [redacted] Monheim, on 14.07.99
Sample: **Winter-rape-seeds Lirajet**
Lab-No.: **1999PR00261**
Active Ingredient: **NTN 33893 / Thiram**

Content Thiram (a.i.) for VAR 1 based on 2 uncounted weights

Sample No.	Seed mass [mg]	Content a.i. [mg]	Content a.i. wt [%]	Content a.i. [g/100kg]
1	12219,2	44,82	0,367	366,80
2	12634,8	45,28	0,358	358,38
mean	***12427,0***	***45,05***	***0,363***	***362,59***

Content NTN 33893 (a.i.) for VAR 2 based on 2 uncounted weights

Sample No.	Seed mass [mg]	Content a.i. [mg]	Content a.i. wt [%]	Content a.i. [g/100kg]
1	12588,3	131,90	1,048	1047,80
2	13048,2	137,60	1,055	1054,55
mean	***12818,3***	***134,75***	***1,051***	***1051,17***

[redacted]

16.7.99

Appendix 9 GLP Certificate of testing facility

[redacted]

Appendix 10 Copy of the Report: Determination of Residues of Imidacloprid and Relevant Metabolites in Nectar, Pollen and Honey of Winter Rape

Bayer AG
Crop Protection Development
Institute for Metabolism Research
and Residue Analysis
D-51368 Leverkusen

2001-03-30
Report No.: MR-147/01

STUDY TITLE

Determination of Residues of Imidacloprid and Relevant Metabolites in Nectar, Pollen and Honey of Winter Rape

Amended by Amendment No. 1 from 2002-03-22

Author

[redacted]

Testing Facility

Bayer AG
PF-E/MR, Building 6610
51368 Leverkusen, Germany

Study Completion Date

2001-04-02

Study Number

E 370 1887-4

MR-147/01 / MO-02-004997

CERTIFICATION OF AUTHENTICITY

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Inquiries should be directed to

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Germany
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[redacted]
Bayer AG
PF-E/MR
51368 Leverkusen
Germany
[redacted]

STATEMENT OF COMPLIANCE

This study was conducted in compliance with the Principles of Good Laboratory Practice (Chemikaliengesetz, dated 25th July 1994, current version of Anhang 1 and the current OECD Principles of Good Laboratory Practice (GLP)).

The Test facility has been inspected and certified as working in compliance with the Principles of Good Laboratory Practice by the competent authorities (Aktenzeichen IV C 4-31.11.62.03, 4th March 1999).

Signature:

[redacted]

Referat GLP
Quality Assurance Statement
[redacted]

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1 INTRODUCTION

Rape flowers, pollen, nectar and honey samples obtained from a German trial station were analysed for residues of Imidacloprid and its olefin- and Hydroxy metabolites. The results are summarized in the table below. Extraction, sample clean-up and determination of Imidacloprid, Hydroxy- and Olefin-metabolite by HPLC-MS/MS were performed according to method 00537/E001 (MR-568/99). The limit of quantitation was 0.005 mg/kg for Imidacloprid and the Hydroxy-metabolite and 0.01 mg/kg for the Olefin-metabolite. The limit of detection was 0.0015 mg/kg for Imidacloprid and the Hydroxy-metabolite and 0.003 mg/kg for the Olefin-metabolite.

2 TIME SCHEDULE

The experimental work was performed during the following time period:

Signature of analytical study protocol: 2000-07-13
Start of analytical phase: 2000-08-01
End of analytical phase: 2000-08-09

3 SAMPLE LIST

3.1 Untreated Plot Samples

The following samples were collected from the honeycombs of the untreated plot:

Sample Material	Sample Origin	Date of Sampling
Pollen (from the Comb)	4 (Colony 114)	2000-05-02
Nectar (from the Comb)	5 (Colony 114)	2000-05-02
Nectar (from the Comb)	7 (Colony 86)	2000-05-02
Nectar (from the Comb)	9 (Colony 72)	2000-05-02
Pollen (from the Comb)	19 (Colony 114)	2000-05-08
Pollen (from the Comb)	20 (Colony 86)	2000-05-08
Pollen (from the Comb)	21 (Colony 72)	2000-05-08
Nectar (from the Comb)	22 (Colony 114)	2000-05-08
Nectar (from the Comb)	23 (Colony 86)	2000-05-08
Nectar (from the Comb)	24 (Colony 72)	2000-05-08
Pollen (from the Comb)	28 (Colony 114)	2000-05-15
Pollen (from the Comb)	30 (Colony 86)	2000-05-15
Pollen (from the Comb)	32 (Colony 72)	2000-05-15
Nectar (from the Comb)	29 (Colony 114)	2000-05-15
Nectar (from the Comb)	31 (Colony 86)	2000-05-15
Nectar (from the Comb)	33 (Colony 72)	2000-05-15
Honey (from the Comb)	34 (Colony 15,90,76)	2000-05-22

3.2 *Treated Plot Samples (Poncho 80 & 420 FS)*

The following samples were collected from the honeycombs of the treated plot:

Sample Material	Sample Origin	Date of Sampling
Nectar (from the Comb)	6 (Colony 126)	2000-05-02
Nectar (from the Comb)	8 (Colony 69)	2000-05-02
Nectar (from the Comb)	10 (Colony 108)	2000-05-02
Pollen (from the Comb)	19 (Colony 126)	2000-05-08
Pollen (from the Comb)	20 (Colony 69)	2000-05-08
Pollen (from the Comb)	21 (Colony 108)	2000-05-08
Nectar (from the Comb)	22 (Colony 126)	2000-05-08
Nectar (from the Comb)	23 (Colony 69)	2000-05-08
Nectar (from the Comb)	24 (Colony 108)	2000-05-08
Pollen (from the Comb)	28 (Colony 126)	2000-05-15
Pollen (from the Comb)	30 (Colony 69)	2000-05-15
Pollen (from the Comb)	32 (Colony 108)	2000-05-15
Nectar (from the Comb)	29 (Colony 126)	2000-05-15
Nectar (from the Comb)	31 (Colony 69)	2000-05-15
Nectar (from the Comb)	33 (Colony 108)	2000-05-15
Honey (from the Comb)	34 (Colony 19,124,35)	2000-05-22

3.3 *Samples from the Honeycombs and Flowers from the Untreated and Treated Plot*

The following samples were collected from the honeycombs and from the flowers of the untreated and treated plot:

Treatement	Sample Material and Origin	Date of Sampling
Untreated		
	Nectar (from the Flowers)	2000-05-04
	Nectar (from the Flowers)	2000-05-05
	Pollen, Swarm 72 (from the Comb)	2000-05-05
	Pollen, Swarm 86 (from the Comb)	2000-05-05
	Pollen, Swarm 114 (from the Comb)	2000-05-05
	Pollen, Swarm 86 (from the Comb)	2000-05-02
	Pollen, Swarm 72 (from the Comb)	2000-05-02
Treated		
	Nectar (from the Flowers)	2000-05-04
	Pollen, Swarm 69 (from the Comb)	2000-05-05
	Pollen, Swarm 126 (from the Comb)	2000-05-05
	Pollen, Swarm 108 (from the Comb)	2000-05-05
	Pollen, Swarm 69 (from the Comb)	2000-05-02
	Pollen, Swarm 108 (from the Comb)	2000-05-02
	Pollen, Swarm 126 (from the Comb)	2000-05-02

3.4 Pooled Samples (Untreated)

The following samples from the honeycombs of the untreated plot were pooled before the analytical determination:

Sample Material	Sample Origin	Date of Sampling	Sample Number
Nectar (from the Comb)	5 (Colony 114)	2000-05-02	7
	7 (Colony 86)	2000-05-02	
	9 (Colony 72)	2000-05-02	
Pollen (from the Comb)	19 (Colony 114)	2000-05-08	8
	20 (Colony 86)	2000-05-08	
	21 (Colony 72)	2000-05-08	
Nectar (from the Comb)	22 (Colony 114)	2000-05-08	9
	23 (Colony 86)	2000-05-08	
	24 (Colony 72)	2000-05-08	
Pollen (from the Comb)	28 (Colony 114)	2000-05-15	10
	30 (Colony 86)	2000-05-15	
	32 (Colony 72)	2000-05-15	
Nectar (from the Comb)	29 (Colony 114)	2000-05-15	11
	31 (Colony 86)	2000-05-15	
	33 (Colony 72)	2000-05-15	
Honey (from the Comb)	34 (Colony 15,90,76)	2000-05-22	12

3.5 Pooled Samples (Poncho 80 & 420 FS)

The following samples from the honeycombs of the treated plot were pooled before the analytical determination:

Sample Material	Sample Origin	Date of Sampling	Sample Number
Nectar (from the Comb)	6 (Colony 126)	2000-05-02	1
	8 (Colony 69)	2000-05-02	
	10 (Colony 108)	2000-05-02	
Pollen (from the Comb)	19 (Colony 126)	2000-05-08	2
	20 (Colony 69)	2000-05-08	
	21 (Colony 108)	2000-05-08	
Nectar (from the Comb)	22 (Colony 126)	2000-05-08	3
	23 (Colony 69)	2000-05-08	
	24 (Colony 108)	2000-05-08	
Pollen (from the Comb)	28 (Colony 126)	2000-05-15	4
	30 (Colony 69)	2000-05-15	
	32 (Colony 108)	2000-05-15	
Nectar (from the Comb)	29 (Colony 126)	2000-05-15	5
	31 (Colony 69)	2000-05-15	
	33 (Colony 108)	2000-05-15	
Honey (from the Comb)	34 (Colony 19,124,35)	2000-05-22	6

3.6 *Pooled Samples (Untreated and Treated Samples)*

The following samples from the untreated and treated plot were pooled before the analytical determination:

Treatement	Sample Material	Date of Sampling	Sample Number
Untreated			
	Nectar (from the Flowers)	2000-05-04	17
	Nectar (from the Flowers)	2000-05-05	
	Pollen, Swarm 72 (from the Comb)	2000-05-05	13
	Pollen, Swarm 86 (from the Comb)	2000-05-05	
	Pollen, Swarm 114 (from the Comb)	2000-05-05	
	Pollen, Swarm 86 (from the Comb)	2000-05-02	14
	Pollen, Swarm 72 (from the Comb)	2000-05-02	
Treated			
	Nectar (from the Flowers)	2000-05-04	18
	Pollen, Swarm 69 (from the Comb)	2000-05-05	15
	Pollen, Swarm 126 (from the Comb)	2000-05-05	
	Pollen, Swarm 108 (from the Comb)	2000-05-05	
	Pollen, Swarm 69 (from the Comb)	2000-05-02	16
	Pollen, Swarm 108 (from the Comb)	2000-05-02	
	Pollen, Swarm 126 (from the Comb)	2000-05-02	

3.7 *Flower Samples*

The following flower samples were analysed:

Sample Material	Sample	Date of Sampling	Sample Number
Flowers	F2233	2000-05-11	1
Flowers	F2234	2000-05-11	2
Flowers	F2235	2000-05-11	3
Flowers	F2236	2000-05-11	4

4 RESULTS

4.1 Results from the Pooled Nectar, Pollen, and Honey Samples from the Untreated and Treated Study Plot

	Sample Material	Number of Pooled Samples	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]	Imidacloprid [mg/kg]
Untreated	Nectar (from the Comb)	7	n.d.	n.d.	n.d.
	Pollen (from the Comb)	8	n.d.	n.d.	n.d.
	Nectar (from the Comb)	9	n.d.	n.d.	n.d.
	Pollen (from the Comb)	10	n.d.	n.d.	n.d.
	Nectar (from the Comb)	11	n.d.	n.d.	n.d.
	Honey (from the Comb)	12	n.d.	n.d.	n.d.
Treated	Nectar (from the Comb)	1	n.d.	n.d.	< LOQ
	Pollen (from the Comb)	2	n.d.	n.d.	n.d.
	Nectar (from the Comb)	3	n.d.	n.d.	< LOQ
	Pollen (from the Comb)	4	n.d.	n.d.	n.d.
	Nectar (from the Comb)	5	n.d.	n.d.	n.d.
	Honey (from the Comb)	6	n.d.	n.d.	n.d.

Limit of quantitation: 0.005 mg/kg for Imidacloprid and Hydroxy-metabolite, 0.01 mg/kg for the Olefin-metabolite
< 0.005 and < 0.010 = Residues below the limit of quantitation (< LOQ)
Limit of detection: 0.0015 mg/kg for Imidacloprid and Hydroxy-metabolite, 0.003 mg/kg for the Olefin-metabolite,
n.d.: Residues below the limit of detection

4.2 Results from Single Analysis of Nectar Samples from the Treated Study Plot

In two of the analysed pooled samples from the treatment plot, residues below the LOQ were detected. To confirm the residue analytical results the single samples were analysed again.

	Sample Material	Number of Pooled Samples	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]	Imidacloprid [mg/kg]
Treated	Nectar (from the Comb)	1/6	n.d.	n.d.	n.d.
	Nectar (from the Comb)	1/8	n.d.	n.d.	< LOQ
	Nectar (from the Comb)	1/10	n.d.	n.d.	< LOQ
	Nectar (from the Comb)	3/22	n.d.	n.d.	< LOQ
	Nectar (from the Comb)	3/23	n.d.	n.d.	< LOQ
	Nectar (from the Comb)	3/24	n.d.	n.d.	< LOQ

Limit of quantitation: 0.005 mg/kg for Imidacloprid and Hydroxy-metabolite, 0.01 mg/kg for the Olefin-metabolite
< 0.005 and < 0.010 = Residues below the limit of quantitation (< LOQ)
Limit of detection: 0.0015 mg/kg for Imidacloprid and Hydroxy-metabolite, 0.003 mg/kg for the Olefin-metabolite,
n.d.: Residues below the limit of detection

4.3 Results from Nectar and Pollen Samples of the Untreated and Treated Study Plot

	Sample Material	Number of Pooled Samples	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]	Imidacloprid [mg/kg]
Control	Nectar (from the Flowers)	17	n.d.	n.d.	n.d.
	Pollen (from the Comb)	13	n.d.	n.d.	n.d.
	Pollen (from the Comb)	14	n.d.	n.d.	< LOQ*
Treated	Nectar (from the Flowers)	18	n.d.	n.d.	n.d.
	Pollen (from the Comb)	15	n.d.	n.d.	n.d.
	Pollen (from the Comb)	16	n.d.	n.d.	n.d.

*: There was not enough sample material to repeat the analysis
Limit of quantitation: 0.005 mg/kg for Imidacloprid and Hydroxy-metabolite, 0.01 mg/kg for the Olefin-metabolite
< 0.005 and < 0.010 = Residues below the limit of quantitation (< LOQ)
Limit of detection: 0.0015 mg/kg for Imidacloprid and Hydroxy-metabolite, 0.003 mg/kg for the Olefin-metabolite,
n.d.: Residues below the limit of detection

4.4 *Results from Flower Samples:*

Sample Number	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]	Imidacloprid [mg/kg]
1	n.d	n.d	n.d
2	n.d	n.d	n.d
3	n.d	n.d	n.d
4	n.d	n.d	n.d

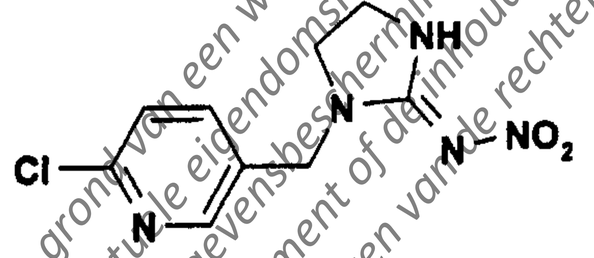
Limit of quantitation: 0.005 mg/kg for Imidacloprid and Hydroxy-metabolite, 0.01 mg/kg for the Olefin-metabolite
< 0.005 and < 0.010 = Residues below the limit of quantitation (< LOQ)
Limit of detection: 0.0015 mg/kg for Imidacloprid and Hydroxy-metabolite, 0.003 mg/kg for the Olefin-metabolite,
n.d.: Residues below the limit of detection

5. EXPERIMENTAL

5.1 Reference Substances

Imidacloprid

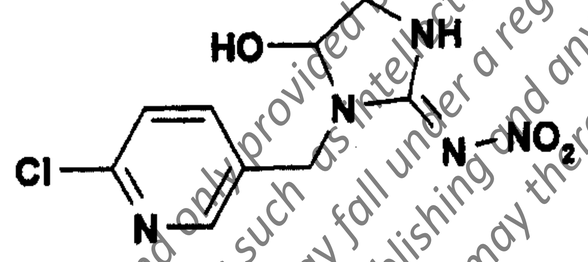
Structural formula:



Empirical formula:	$C_9H_{10}ClN_5O_2$
Molecular weight:	255.7 g/mole
Certificate of analysis:	M06693, 2000-01-11
Certified assay:	99.8 %
Expiry date:	November 2001

Hydroxy-Imidacloprid (WAK 4103)

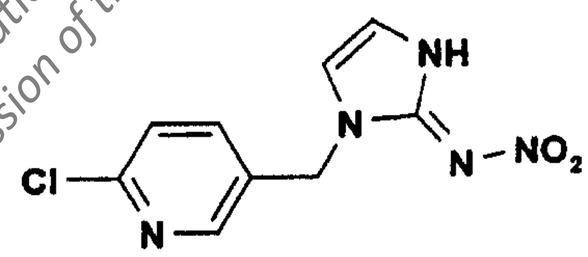
Structural formula:



Empirical formula:	$C_9H_{10}ClN_5O_4$
Molecular weight:	271.7 g/mole
Certificate of analysis:	930323ELB03, 2000-05-11
Certified assay:	99.4 %
Expiry date:	May 2005

Olefin-Imidacloprid (NTN 35884)

Structural formula:



Empirical formula:	$C_9H_8ClN_5O_2$
Molecular weight:	253.6 g/mole
Certificate of analysis:	M11453, 2000-07-28
Certified assay:	98.6 %
Expiry date:	July 2002

5.2 *Residue Analytical Methodology*

5.2.1 *Extraction and Sample Clean-up*

1. Place for e.g. 2.0 g of the sample material in a 150-mL beaker.
   Add 30 mL of methanol/water (3/1,v/v) and allow the sample to soak for 30 min.
2. Blend the sample using an ultra-turrax blender (or equivalent) for approximately 1 min.
3. Vacuum filter the suspension through 2.5 g of Celite filter aid using Schwarzband filter paper supported on a Büchner funnel into a 250-mL vacuum filter flask.
4. Wash the filtered solids with a total of 30 mL of methanol/water (3/1, v/v). Press residual solvent from the solids using rubber damming. Discard the filtered solids.
5. Transfer the filtrate to a 100-mL graduated cylinder. Determine the total volume of the extracts. Mix the solution well, and transfer the half (e.g. 1.0 g sample equivalent) to a 250-mL brown glass round-bottomed flask.
6. Concentrate the aliquot to an aqueous remainder of 5 to 10 mL using a rotary evaporator with a max. bath temperature of 50 °C.

5.2.2 *ChemElut® Column Clean-up*

1. Add 5 to 10 mL water to the aqueous solution from 5.2.1 step 6 to bring the total volume of the extracts to approx. 20 mL.
2. Place the aqueous solution on the top of the ChemElut® CE 1020 (20 mL volume) column fitted with a disposable stainless steel needle and wait for approx. 15 minutes to achieve an uniform distribution of the liquid on the column.
3. Elute the residues from the column with 140 mL of CH_2Cl_2. Collect the eluate in a 250-mL brown glass round-bottomed flask.
4. Evaporate the eluate from step 3 to dryness using a vacuum rotary evaporator and a max. bath temperature of 40 °C.

5.2.3 Silica Gel Column Clean-up

1. Dissolve the residues from 5.2.2 step 4 in 2 mL of toluene/ethyl acetate (85/15, v/v).

2. Apply the organic solution from step 1 onto a 0.5 g (3 mL) silica gel (SiOH) column (e.g. Varian).

3. Allow the solution to pass through the column at a flow rate of 1 mL/min.

4. Rinse the 250-mL brown glass round-bottomed flask with 10 mL of toluene/ethyl acetate (70/30, v/v) and apply the solution onto the column, too.

5. Elute the residues with 5 mL of acetonitrile at a flow rate of 1 mL/min. Collect the eluate in a 25-mL brown glass pear-shaped flask.

6. Evaporate the eluate from step 5 to dryness using a vacuum rotary evaporator and a max. bath temperature of 40 °C. Dissolve the residues in e.g. 1.00 mL of acetonitrile/water (2/8, v/v) and determine the residues with HPLC-MS/MS.

NOTE

1. **The volumes to be used for flushing the column with toluene/ethyl acetate and for elution with acetonitrile must be newly determined for each batch of SiOH-column!**

2. **The flow rate should not be too high, since otherwise losses of the residues in may occur with recoveries below 70 % and the clean-up is less effective.**

3. **The Hydroxy-Metabolite may be converted to the Olefin-Metabolite (especially under acidic conditions).**

4. **The Olefin-Metabolite is degraded by light (ca. 50% in one day at natural daylight). Therefore, all solutions containing the Olefin-Metabolite must be protected from light and stored in a cool and dark place.**

5.3 *HPLC-MS/MS Determination of Imidacloprid and Metabolites*

5.3.1 *Measuring Equipment and HPLC Conditions:*

Instrument:	Hewlett Packard 1100
Column:	e.g.: Phenomenex, Luna C18 (2), 5 µm, 15 x 0.46 cm i.D. or Merck, Superspher, RP select-B, 4 µm, 12.5 x 0.4 cm i.D.
Solvent A:	Water/ACN (9/1, v/v) + 0.1 mL Acetic acid/L
Solvent B:	ACN + 0.1 mL Acetic acid/L
Oventemperature:	40 °C
Inject.volume:	50 µL
Flow:	1.0 mL/min
Split:	150 µL into MS from 1000 µL

Time Table	0 min	11.1 % B TO MS
	10 min.	11.1 % B
	10.1 min	90 % B
	11 min	90 % To Waste
	15 min	90 % B
	15.1 min	11.1 % B
	16 min	11.1 % B TO MS
	19 min	11.1 % B

Retention Times:	Olefin-Imidacloprid	approx. 4.8 min
	Hydroxy-Imidacloprid	approx. 5.6 min
	Imidacloprid	approx. 8.5min

5.3.2 *MS/MS-Detection*

The experiments were performed on a triple-quadrupole mass spectrometer fitted with an electrospray interface operated in the positive ion mode under MRM conditions.The mass spectrometer was tuned by infusing a standard solution of 0.5 mg/L Imidacloprid, Hydroxy-Metabolite and Olefin-Metabolite (dissolved in acetonitrile / water (2/8, v/v) + 0.1 mL acetic acid per litre) at a flow rate of 5-10 µL/min. Mass axis calibration was done by infusing a polypropylene glycol solution. Unit mass resolution was established and maintained in each mass resolving quadrupole by maintaining a full widht at half-maximum of between 0.8 and 1.0 DA. After tuning and calibration, optimal collision-activated dissociation (CAD) conditions for fragmentation of Imidacloprid, Hydroxy-Metabolite and Olefin-Metabolite were determined. These experiments were performed with nitrogen as collision gas with a collision offset of -19 and -23 eV for Imidacloprid, -23 eV for Hydroxy-Metabolite and –12 and -13 eV for Olefin-Metabolite at an approximate collision gas thickness of 1.56×10^{15} atoms/cm^2. Nebulization gas is set at 1.48 L/min, curtain gas is set at 1.44 L/min, CAD gas is set at 0.87 L/min and turbo gas is set at 7 L/min.

Detector:	Triple Quadrupol LC-MS/MS Mass Spectrometer PE Biosystems (Perkin-Elmer Sciex Instruments) API 365, Windows NT 4.0 System
Interface:	Electrospray, Turbo Ion Spray Potential: + 4400 V Temperature: 400° C (Source)
Gas:	Nebulization gas: 1.48 L/min (liquid nitogen 5.0) Curtain gas: 1.44 L/min (liquid nitogen 5.0) Collision gas 0.87 L/min (liquid nitogen 5.0) Turbo gas: 7 L/min (liquid nitogen 5.0)
Scan Type:	MRM (Multiple Reaction Monitoring Mode)
Polarity:	Positive
Aquisition mode:	Profile

Mass spectrometer operating parameters

Compound	Precursor Ion Q1 Mass (amu)	Product Ion Q3 Mass (amu)	Dwell Time (msec)	Collision Energy (eV)
Imidacloprid (Cl 37)#	258	211	250	-19
Imidacloprid (Cl 35)	256	209	250	-19
Imidacloprid (Cl 37)#	258	175	250	-23
Imidacloprid (Cl 35)	256	175	250	-23
Hydroxy-Metabolite (Cl 37)#	274	191	250	-23
Hydroxy-Metabolite (Cl 35)	272	191	250	-23
Olefin-Metabolite (Cl 35)#	254	236	250	-12
Olefin-Metabolite (Cl 35)	254	207	250	-13

#= ^{37}Cl isotope of all substances were detected to use as qualifiers

Bayer AG
Crop Protection Development
Institute for Metabolism Research
and Residue Analysis
D-51368 Leverkusen

STUDY TITLE

Determination of Residues of Imidacloprid and Relevant Metabolites in Nectar, Pollen and Honey of Winter Rape

Amendment

Amendment No. 1 from 2002-03-22

Author of the Amendment

[redacted]

Testing Facility

Bayer AG
PF-E/OE, Building 6620
51368 Leverkusen, Germany

Study Number

E 370 1887-4

Reason for Amendment

The rape variety used in this study was erroneously described as Summer Rape while it was Winter Rape.

Remark:

The corresponding pages 1 and 4 have been exchanged.

[redacted]

Study Director 2002-03-22
Date

Manager of Test Facility (PF-E/OE) and Representative of the Sponsor 22.3.02
Date

Quality assurance statement to Amendment 1

Referat GLP
Quality Assurance Statement
[redacted]