

Study Title

Evaluation of the effects of residues of Imidacloprid FS 600 in maize pollen from dressed seeds on honeybees (*Apis mellifera*) in the semifield
Appendix XIII containing data from Report MR-547/01

Guideline

special design, no standard guideline available

Author

[REDACTED]

Study Completion date

2002-03-07

Performing Laboratory

Bayer AG
Crop Protection
Institute for Environmental Biology
51368 Leverkusen
Germany

Laboratory Project ID

E 319 2046 - 5



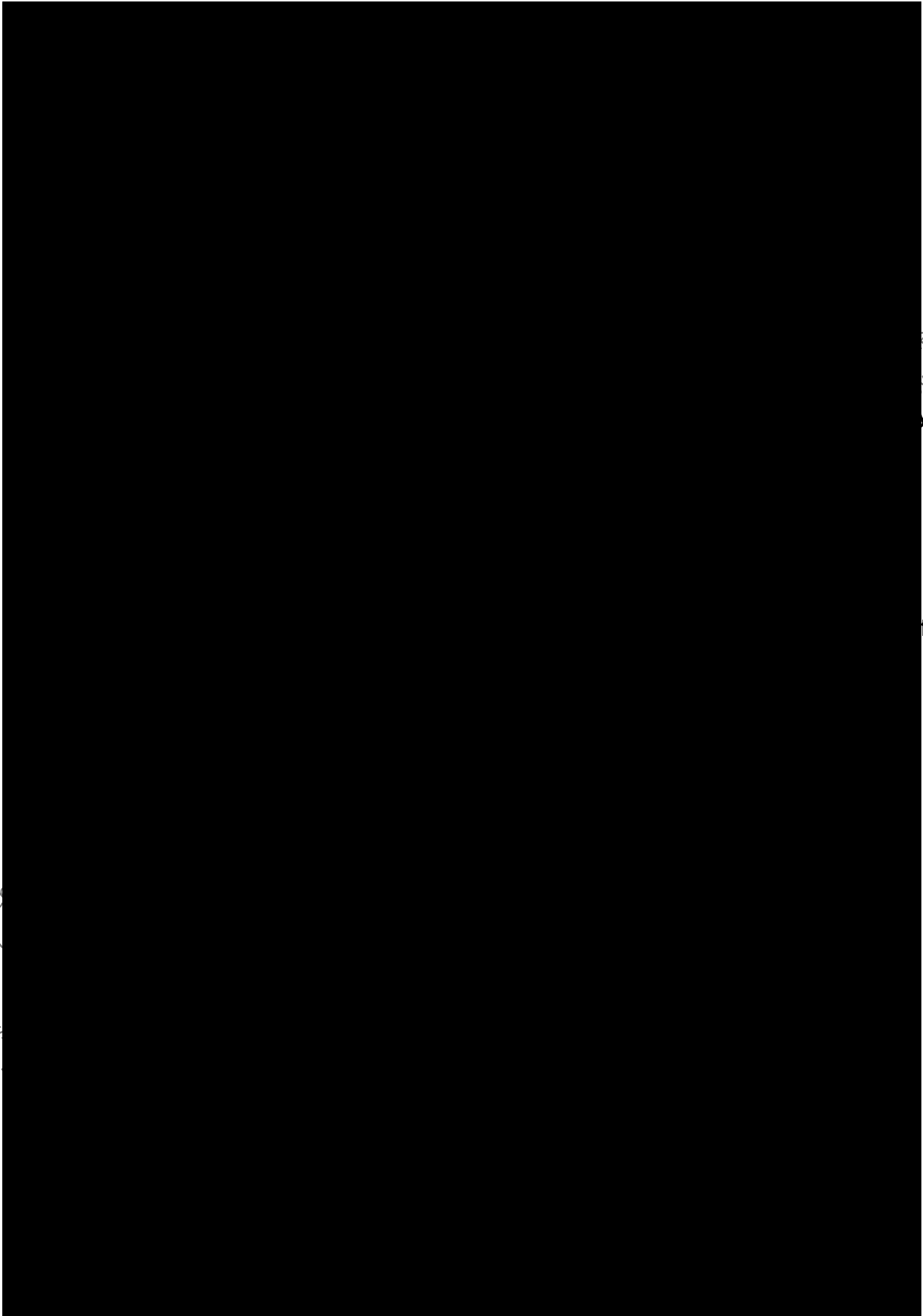
Data Confidentiality Statement

This page is intentionally left blank for the purpose of submitting administrative information that is required by regulations promulgated by various countries.

*Dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt op grond van een wettelijke verplichting tot openbaarmaking.
Op dit document kunnen rechten van derden rusten, waaronder intellectuele eigendomsrechten en/of auteursrechten.
Voorts kan dit document onder een regeling omtrent gegevensbescherming vallen.
Publicatie, verspreiding, vermenigvuldiging, commerciële exploitatie en gebruik van dit document of de inhoud hiervan zonder de toestemming van de rechthebbende van dit document kan derhalve verboden zijn en een inbreuk opleveren van de rechten van deze rechthebbende.*

*This document is not the property of the Ctgb and only provided based on mandatory freedom of information requirements.
The document may be subject to rights such as intellectual property and copy rights of third parties.
Consequently, any publication, distribution and/or publishing and any commercial exploitation and use of this document or its contents without the permission of the owner of this document may therefore be prohibited and violate the rights of its owner.*

Certification of Good Laboratory Practice

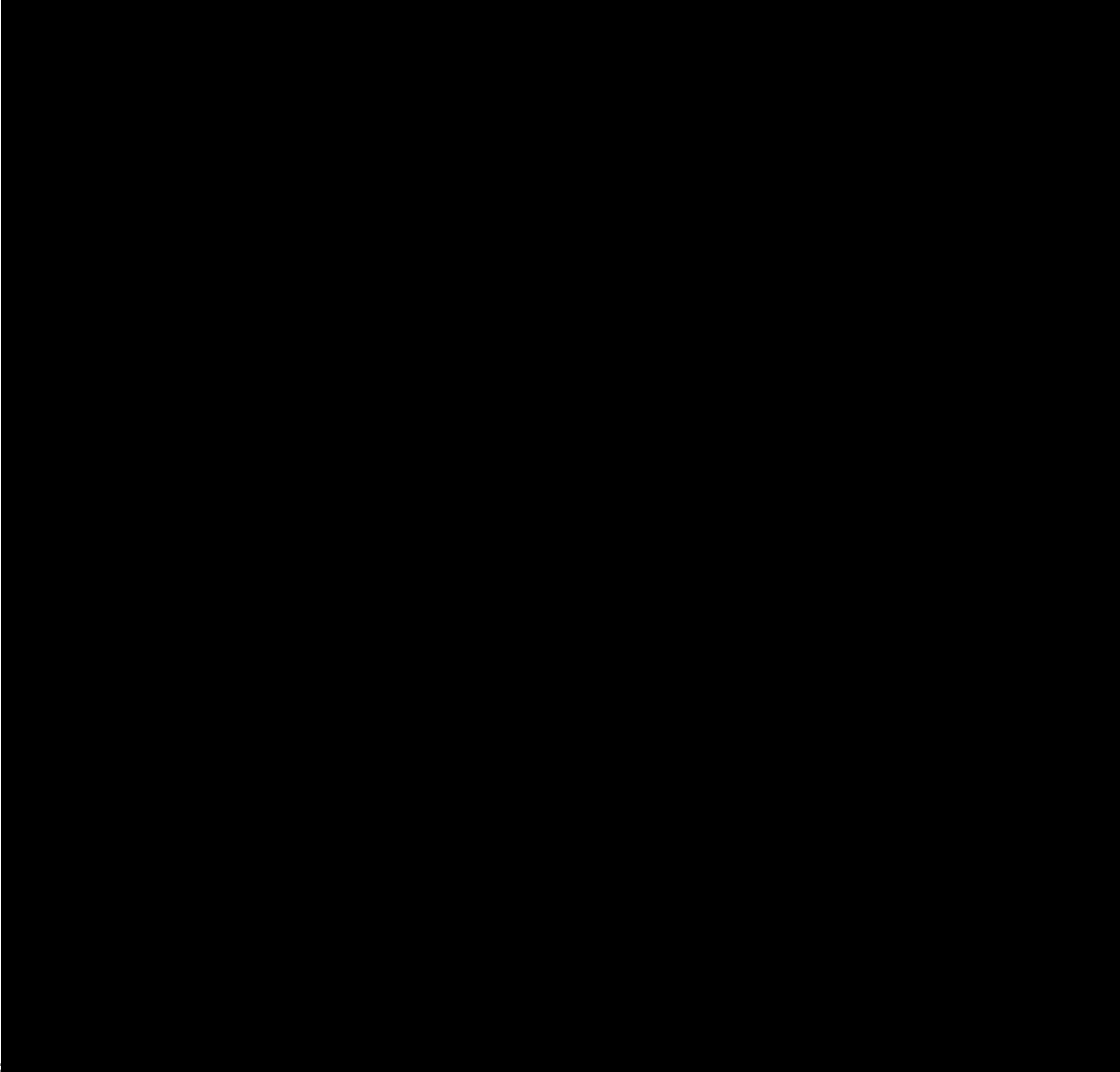


Dit document is geë
Op dit
Publicatie, vers

ent or its contents
wner.

f

Certification of Authenticity



Dit document is
Op dit
Publicatie, verspreiding, ver
rechthebbende va
This document is not the prop
The document may
Furthermore,
Consequently, any publication, distribution, repro
without the permission of the owner

... of its contents
ner.

Quality Assurance Statement

Referat GLP
Quality Assurance Statement

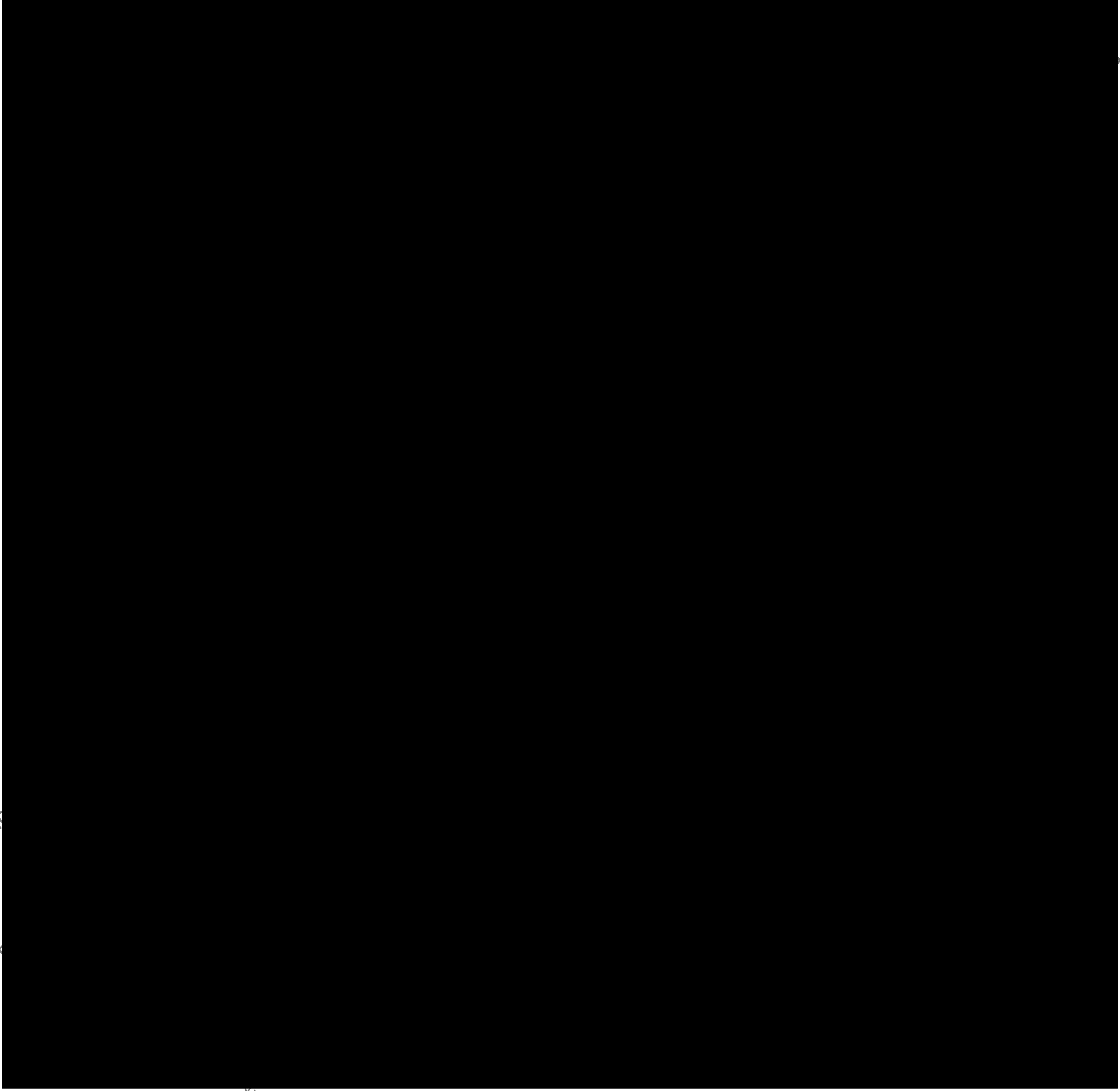


TABLE OF CONTENTS

	Page No.
Data Confidentiality Statement.....	2
Certification of Good Laboratory Practice	3
Certification of Authenticity	4
Quality Assurance Statement.....	5
1.0 SUMMARY	8
2.0 INTRODUCTION	11
3.0 EXPERIMENTAL.....	11
3.1 Test Substance, Control, Reference	11
3.1.1 Test Substance	11
3.1.2 Pollen from treated maize plants.....	11
3.1.3 Control	11
3.1.4 Reference	11
3.2 Execution of the Test.....	12
3.3 Origin of Honeybees and Preparation of Hive Colonies.....	12
3.4 Preparation of the Food Pollen.....	12
3.5 Location of the Trial Site and Description of the Study Plots	13
3.6 Treatment Design	13
3.7 Climatic Conditions During the Study.....	15
3.8 Observations on Honeybees Colonies.....	16
4.0 FILING	18
5.0 AMENDMENTS TO THE STUDY PLAN	18
6.0 RESULTS AND DISCUSSION.....	19
6.1 Climatic Conditions During the Study.....	19
6.2 Biological Observations on the foraging Honeybees.....	19
6.3 Population Development and Breeding Performance.....	29
6.4 Summary of the Analytical Findings of grown residues of Imidacloprid FS 600 in pollen	33
7.0 CONCLUSION.....	33
APPENDICES	
APPENDIX I: Climatic Conditions as Recorded During Evaluation Dates	34
APPENDIX II: Mortality in Control and Treatment.....	35
APPENDIX III: Comb Production in Control and Treatment.....	37
APPENDIX IV: Quantity of Honey and Pollen collected by the Foraging Honeybees in Control and Treatment.....	38
APPENDIX V: Size of Honey Stores over Time in Control and Treatment.....	40
APPENDIX VI: Size of Pollen Stores over Time in Control and Treatment.....	41
APPENDIX VII: Weight increase of bee hives in Control and Treatment	42
APPENDIX VIII: Queen Egg Laying Activity in Control and Treatment	43
APPENDIX IX: Abundance of Honeybee Larvae (non-capped brood) in Control and Treatment..	44
APPENDIX X: Abundance of Honeybee Pupae (capped brood) in Control and Treatment.....	45
APPENDIX XI: Population Development in Control and Treatment	46
APPENDIX XII: Activity Pattern of Foraging Honeybees in Control and Treatment.....	47
APPENDIX XIII: Analytical Report - Evaluation of the Effects of Residues of Imidacloprid FS 600 in Maize Pollen from Dressed Seeds on Honeybees (Apis Mellifera) in the Semifield	50

TABLES

Table 1: Summary 9

Table 2: Allocation of the bee colonies to the tunnels 13

Table 3: Replacement of the pollen feeders 14

Table 4: Records of climatic conditions 15

Table 5: Mortality of the bees in front of the bee hive and at the tunnel edges 19

Table 6: Area of comb cell production in cm² 21

Table 7: Honey consumption 22

Table 8: Pollen consumption 23

Table 9: Comb area with honey stores in cm² comb area 24

Table 10: Comb area with pollen stores in cm² comb area 25

Table 11: Weight development of the bee hives 26

Table 12: Foraging activity of the honeybees 27

Table 13: Comb area with cells containing eggs in cm² comb area 29

Table 14: Comb area with cells containing larvae in cm² comb area 30

Table 15: Comb area with cells containing pupae in cm² comb area 31

Table 16: Comb area covered with adult honeybees during assessments in cm² 32

FIGURES

Figure 1: Mortality of the bees in front of the bee hive and at the tunnel edges 20

Figure 2: Area of comb cell production in cm² 21

Figure 3: Honey consumption 22

Figure 4: Pollen consumption 23

Figure 5: Comb area with honey stores in cm² comb area 24

Figure 6: Comb area with pollen stores in cm² comb area 25

Figure 7: Weight development of the bee hives 26

Figure 8: Foraging activity of the honeybees 28

Figure 9: Comb area with cells containing eggs in cm² comb area 29

Figure 10: Comb area with cells containing larvae in cm² comb area 30

Figure 11: Comb area with cells containing pupae in cm² comb area 31

Figure 12: Comb area covered with adult honeybees during assessments in cm² 32

Dit document is geen eigendom van het GCB en wordt beschikbaar gemaakt op grond van een wettelijke verplichting tot openbaarmaking.
 Op dit document kunnen rechten van derden rusten, waaronder intellectuele eigendomsrechten en/of auteursrechten.
 Publicatie, verspreiding, vermenigvuldiging, verspreiden van dit document of anderszins openbaar maken van de inhoud hiervan zonder de toestemming van de rechthebbende.
 Consequently, any publication, distribution, reproduction and/or publishing of this document may therefore be prohibited and violate the rights of its owner.

1.0 SUMMARY

Report: [REDACTED] (2002): Evaluation of the effects of residues of Imidacloprid FS 600 in maize pollen from dressed seeds on honeybees (*Apis mellifera*) in the semifield

Bayer AG, unpublished report No: MAUS/Am018; 2002-03-07

Analytical Report: SCHOENING, R. (2001): Evaluation of the effects of residues of Imidacloprid FS 600 in maize pollen from dressed seeds on Honeybees (*Apis mellifera*) in the semifield

Bayer AG, unpublished report No: MR-547/01, 2001-11-28

Guidelines: Internal Testing Method
Deviations: not applicable

GLP: yes (certified laboratory)

Material and methods: Test substance: maize pollen with grown residues of Imidacloprid FS 600 (seeds dressed with commercially available product at a rate of 1 g a.i. /1000 seeds). Small honeybee colonies (approx. 500 honeybees) were confined on oat plots (50 m² drilled on 2001-05-03) in tunnels and fed with maize pollen containing grown residues of Imidacloprid or untreated control pollen. For treatment and control, three replicates were set up each. Sunflower honey was provided as carbohydrate source. The small bee colonies were examined for treatment-related effects over a period of 52 days. In particular, the endpoints mortality and foraging intensity were evaluated. Likewise the endpoints comb cell production, food consumption, pollen and honey stores, egg laying activity, breeding success, colony strength and hive weight development were assessed and statistically analysed using a t-Test. Behavioural anomalies were also assessed.

Dates of biological work: 2001-06-21 to 2001-08-12

Dates of analytical work: 2001-03-14 to 2001-06-05

Honeybee Semifield Test Imidacloprid

Findings: Effects of residues of Imidacloprid FS 600 in pollen on small honeybee colonies

Table 1: Summary

Testing Endpoint	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
Mortality (Total No. of dead bees in front of the bee hives) [n]	1	1	0	5	7	3
Mortality (Total No. of dead bees at the tunnel edge) [n]	28	31	25	50	34	50
Cumulative comb cell production at study termination [cm ²]	768	708	675	692	651	768
Cumulative honey collected [g]	702	694	677	621	661	668
Cumulative pollen collected [g]	12.2	8.9	9.6	21.0	39.8	27.1
Honey storage area at study termination [cm ²]	194	234	116	133	172	226
Pollen storage area at study termination [cm ²]	41	13	18	17	25	27
Egg laying activity [cm ² comb area containing cells with eggs at study termination]	177	98	130	182	125	181
Larval abundance [cm ² comb area containing cells with larvae at study termination]	92	70	79	99	67	120
Pupal abundance [cm ² comb area containing cells with pupae at study termination]	113	92	149	86	110	125
Colony strength [cm ² comb area covered with bees at study termination]	266	183	183	265	222	260
Hive weight increase [%]	25.5	27.6	22.1	17.3	23.6	21.6
Foraging activity [Average No. of bees at the pollen feeder/assessment]	0.7	0.7	0.7	0.8	1.1	0.6
Foraging activity [Average No. of bees at the honey feeder/assessment]	7.3	8.1	7.7	7.3	8.2	7.9

Observations: There were no significant differences between control and treatment in comb cell production ($t=-0.478$, $p=0.641$), honey consumption ($t=2.530$, $p=0.065$), hive weight increase ($t=1.720$, $p=0.161$), pollen stores ($t=-0.360$, $p=0.725$) and honey stores ($t=0.086$, $p=0.933$), egg deposition ($t=-0.176$, $p=0.863$), larval abundance ($t=-0.328$, $p=0.749$), pupal abundance ($t=-0.288$, $p=0.778$) and abundance of adult bees ($t=-0.068$, $p=0.947$). The pollen consumption was significantly higher in the treatment

than in the control ($t=-3.391$, $p=0.027$), which can be explained by the different granulation of the control and treatment pollen. The treatment pollen was finer than the control pollen and was therefore easier to transport by the bees. Foraging activity at the pollen feeder and the honey feeder was comparable in control and treatment. Likewise, mortality was comparable in control and treatment, although slightly higher in the treatment but well in the usual range of bee mortality in both control and treatment. No behavioural anomalies were observed in control or treatment.

The residue levels of Imidacloprid and its Hydroxy-metabolite and Olefin-metabolite determined in pollen which originated from seeds dressed with Imidacloprid FS 600 were below the limit of quantation (Imidacloprid and Hydroxy-metabolite: LOQ=0.005 mg/kg; Olefin-metabolite LOQ=0.01 mg/kg).

Conclusion: The results of the study show that there is no risk to honeybees from foraging on and consuming maize pollen of plants originating from seeds dressed with Imidacloprid FS 600 at the rate of 1 g a.i./1000 seeds.

Dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt op grond van een wettelijke verplichting tot openbaarmaking.
Op dit document kunnen rechten van derden rusten, waaronder intellectuele eigendomsrechten en/of auteursrechten.
Voorts kan dit document onder een regeling omtrent gegevensbescherming vallen.
Publicatie, verspreiding, vermenigvuldiging, commerciële exploitatie en gebruik van dit document of de inhoud hiervan zonder toestemming van de rechthebbende van deze rechten is niet toegestaan.
Consequently, any publication, distribution, reproduction and/or publishing and any commercial exploitation and use of this document or its contents without the permission of the owner of this document may therefore be prohibited and violate the rights of its owner.

2.0 INTRODUCTION

According to EU directive 91/414/EEC the impacts of pesticides on honeybees have to be examined. If laboratory studies indicate a potential hazard to honeybees, higher tier studies are required for a field-relevant risk assessment. The present study aims to examine the effect of maize pollen from plants which seeds had been dressed with Imidacloprid FS 600 on the development of small bee colonies and on behaviour and mortality of honeybees.

3.0 EXPERIMENTAL

3.1 Test Substance, Control, Reference

3.1.1 Test Substance

Test substance:	Imidacloprid FS 600 (= Gaucho)
Active ingredient:	NTN 33893
Chemical name of a.i. (CAS):	2-Imidazolidinimine, 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-
CAS number of a.i.:	138261-41-3
Development number:	not applicable, commercially available product*
Batch number:	not applicable, commercially available product*
TOX-No.:	not applicable, commercially available product*
a.i. contents:	not applicable, commercially available product*
Analytical method:	not applicable, commercially available product*
Date of analysis:	not applicable, commercially available product*
Expiry date:	not applicable, commercially available product*
Storage conditions:	Room temperature
Safety Precaution:	Routine hygienic precautions

* The relevant parameter for this study was the a.i. residue content in the pollen from the treated plants, which was analytically verified.

3.1.2 Pollen from treated maize plants

Maize variety:	Agroceres
Contents of NTN 33893 acc. to analysis:	below limit of detection
Date of analysis:	2001-03-14 to 2001-06-05 (see appendix XIII)
Expiry date:	not applicable, pollen fed immediately after analysis
Storage conditions:	ca. 12-15° C
Safety precautions:	Routine hygienic precautions

3.1.3 Control

Untreated pollen was fed to the bees of the control groups.

3.1.4 Reference

For this type of study, a reference compound is not specified and was thus not applied.

3.2 Execution of the Test

The study field site was drilled with oat on 2001-05-03. Tunnel cages (50 m²) were placed on this oat field on 2001-06-06 and confined the study plots. The hive colonies were placed inside the tunnels on 2001-06-21. The final evaluations on these hives were made on 2001-08-12.

Sponsor:

BAYER AG
PF-E/PBA
D-40789 Monheim

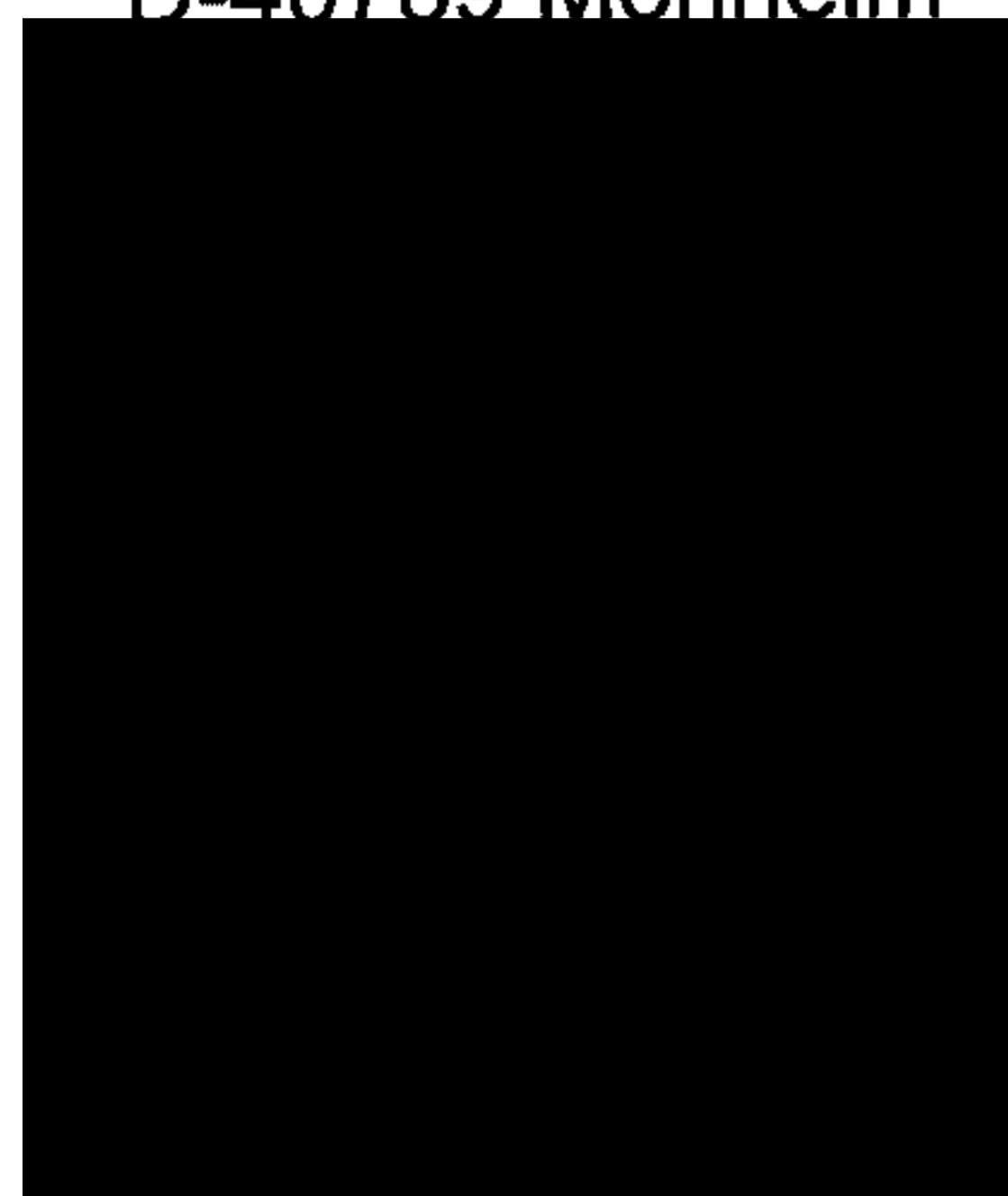
Study director:

Draft report:

Responsible analyst:

Apicultural manager:

Study technicians:



Quality assurance:

PF-BPA / QM-GLP

3.3 Origin of Honeybees and Preparation of Hive Colonies

Honeybees were obtained from a German beekeeper ([REDACTED]). Preparation of the hive colonies used for the test started on 2001-06-20. Honeybees from combs from a large commercially managed beehive were swept down into a drone sieving cage and moistened with water to suppress escape flights. These honeybees were divided into 120 g subsamples, which is equivalent to a number of approx. 500 honeybees. Each subsample was filled into a multiple-comb-fertilisation-cage (= "Mehrwaben-Begattungskästchen") which contained 4 native comb strips (11 x 2 cm), i.e. only comb matrices. One queen in egg laying activity was added to each of these hive colonies within a separate and closed cage, which was removed later. The hives were set up in the tunnels on 2001-06-21 and were taken out of the tunnels on 2001-08-03 and transferred to Schievelsberg (Zülpich-Lövenich) for further evaluations. On 2001-08-06 the bee hives were transferred to Kall.

3.4 Preparation of the Food/Pollen

According to the study plan, pollen originating from South Africa and Brazil was to be used in this feeding test. Due to the growth of fungus on the pollen from South Africa, the maize pollen fed in this study only originated from [REDACTED] (Brazil) and was sent to the testing facility by [REDACTED] (Bayer Brazil) on 2001-02-05 and arrived on 2001-02-09.

The maize plant seeds (variety "Agroceres") were dressed (not under GLP) with commercially available product of Imidacloprid FS 600 at a rate of 100 g a.i. /30 kg seeds. Control seeds received no treatment.

The seeds were sown on 2000-11-23 (not under GLP). The soil of the field was analysed under non-GPL conditions and the soil particles are described as follows: 3.1% coarse sand, 7.3% fine sand, 37.6% clay, 51.9% silt. The pH-value was 4.8, the components of organic material 2.2% and the capacity of cations exchange was 75.

Freshly shot plants were found on 2000-11-28. Ammonium sulphate fertiliser was brought out on the fields at a rate of 100 kg/ha on 2000-12-09 and 2000-12-26. No

treatment with plant protection products occurred during the growing time of the maize plants.

Maize panicles were harvested in Brazil from 2001-01-27 to 2001-01-31, transferred into paper bags and stored in a container (not under GLP). The control pollen was harvested during wet and the treatment pollen during dry weather conditions. Cleaning of the panicles was carried out by sieving the panicles through sieves size 1 and 3. The pollen was dried at the facilities of Bayer Brazil in an oven at a temperature of 60°C for 36 hours from 2001-02-01 to 2001-02-03 (not under GLP). As a result of the harvestment during wet weather conditions the treatment pollen was more pulverulent than the control pollen.

Samples of the pollen were taken for analysis to determine the residue level of the test substance. The analytical results are summarised in chapter 6.4 and reported in appendix XIII. The Imidacloprid- residue level in the pollen samples was below the limit of quantitation; the metabolites Hydroxy-Imidacloprid and Olefin-Imidacloprid of the test substance were not detected.

3.5 Location of the Trial Site and Description of the Study Plots

The trial site was located in the vicinity of Euskirchen-Billig (Germany, Nordrhein-Westfalen). The field was cropped with oat. On 2001-05-30 the study plots were treated with Tristar 1.5 l/ha against the growth of weeds. On 2001-07-10 the oat plants were cut with a motor scythe. In order to prevent honeybees from leaving the test plots and to collect honey or pollen from other sources than the ones offered in the test, six plots were confined with tunnels of 50 m² floorspace (10 x 5 m) on the test field on 2001-06-06. The tunnels consisted of an aluminium frame covered by plastic gauze material (ca. 2 x 2 mm mesh size).

3.6 Treatment Design

After preparation of the bee colonies (see 3.4), each of them was allocated to one of the six tunnel cages using a random list. The bee colonies were transferred into the tunnels on 2001-06-21. The allocation of the colonies to control and treatment was as follows:

Table 2: Allocation of the bee colonies to the tunnels

Bee colony	Tunnel No.	Treatment
5	5	Control, 1a
9	9	Control, 1b
3	3	Control, 1c
1	1	Imidacloprid, 3a
8	8	Imidacloprid, 3a
7	7	Imidacloprid, 3a

Additionally to the pollen, sunflower honey (Honig Müngersdorff, Köln) was offered to the bees as food resource in a feeder placed outside the bee hives. The sunflower honey was provided in elevated and sheltered glass containers which were positioned in a distance of some meters from the hives. The honey was provided in small, weighed portions. Each 1st to each 6th day, a fresh portion was offered and the remaining old portion removed and reweighed. The honey was analysed for background contamination. The analytical results are reported in the appendix XIII of this study (MR 547/01). According to these results, the honey was free of

Imidacloprid background contaminations and background contaminations of the metabolites Hydroxy-Imidacloprid and Olefin-Imidacloprid and of TI 435, TZMU and TZNG, which is documented in the raw data.

The pollen was provided in ca. 8.36-36.60 g portions at two different places (in- and outside the hives). One portion was offered in a separate, sheltered container next to the honey feeder and a second portion was offered in an open glass container placed on the hive bottom. As the sunflower honey, the pollen subsamples were stored during the study within a refrigerator between +6 and +9°C. Pollen portions of the feeders were replaced by fresh portions on the following study days.

Table 3: Replacement of the pollen feeders

Feeder	Fresh Pollen Subsample on Days
Hive feeder	8, 12, 18, 22, 29, 34, 36, 45
Field feeder	8, 15, 22, 29, 36

At each replacement event and finally after termination of the biological part of the study, the amount of collected pollen was determined gravimetrically.

Dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt op grond van een wettelijke verplichting tot openbaarmaking.
 Op dit document kunnen rechten van derden rusten, waaronder intellectuele eigendomsrechten en/of auteursrechten.
 Voorts kan dit document onder een regeling omtrent gegevensbescherming vallen.
 Publicatie, verspreiding, vermenigvuldiging, commerciële exploitatie en gebruik van dit document zijn en een inbreuk opleveren op de rechten van deze rechthebbende.
 This document is not the property of the Ctgb and only provided based on mandatory freedom of information requirements.
 The document may be subject to rights such as intellectual property and copy rights of third parties.
 Furthermore, this document may fall under a regulatory data protection regime.
 Consequently, any publication, distribution and/or publishing and any commercial exploitation and use of this document or its contents without the permission of the owner of this document may therefore be prohibited and violate the rights of its owner.

Honeybee Semifield Test Imidacloprid

3.7 Climatic Conditions During the Study

During the study, temperature and precipitation events were continuously recorded using thermohygrographs and precipitation measuring devices.

The following additional records were made during the evaluation checks using a thermometer and a mobile anemometer (always between 9:50 and 16:30):

Table 4: Records of climatic conditions

Day after first exposure	Air temperature [°C]	Soil temperature [°C]	Precipitation [mm]	Cloudiness (% sky coverage)	Wind speed (estimates)*
0 (n.r.)	n.r.	n.r.	n.r.	n.r.	n.r.
1 (12:00-14:00)	21	22	0	60	+++
2 (11:15-11:55)	13	15	0	100	+
4 (13:50-14:45)	25	30	0	30	++
5 (11:00-12:20)	26	29	0	0	+
6 (11:00-12:20)	27	32	0	0	+
7 (14:00-15:20)	22	24	12	80	+
8 (13:00-14:20)	24	26	0	85	+
11 (12:20-13:40)	20	20	13	95	+
12 (11:50-13:10)	22	24	0	0	++
13 (10:30-11:50)	24	24	0	0	++
14 (13:30-14:50)	29	34	0	0	+
15 (12:50-14:10)	26	32	0	0	+
18 (15:00-16:20)	21	23	8	80	++
19 (9:50-11:10)	21	24	0	80	++
20 (13:00-15:00)	18	22	3	100	++
21 (15:10-16:30)	18	18	1	100	++
22 (11:30-12:50)	18	20	1	90	++
25 (14:20-15:40)	19	22	20	50	+
26 (11:30-12:50)	21	26	0	80	+
27 (14:00-15:20)	19	22	2	70	+++
28 (13:00-14:20)	18	22	0	80	+++
32 (10:30-11:50)	24	30	3	0	+
33 (10:20-11:40)	22	27	0	80	++
34 (12:30-13:50)	27	30	0	40	+
35 (11:00-12:20)	25	27	0	0	n.r.
36 (13:00-14:20)	28	30	0	0	n.r.
39 (13:30-14:50)	28	30	0	50	+
40 (12:00-13:20)	29	30	0	15	++
41 (13:20-14:40)	21	24	0	10	+
42 (n.r.)	29	31	0	0	+

- = calm, + = slight wind, ++ = moderate wind velocity, +++ = high wind velocities, stormy, n.r. = not reported

3.8 Observations on Honeybees Colonies

All anomalies in the development and behaviour of the exposed honeybee colonies were recorded together with the dates of observation. In particular, the following behavioural endpoints were evaluated:

- Mortality:** In front of the colony hives and at the tunnel edges cotton sheets of 60 x 50 cm were spread on the ground. Dead bees were collected from these sheets daily except during weekends and the data recorded. Mortality was compared between the control and treatment based on the total average number of bees found dead per treatment.
- Comb cell production:** The increase in the comb cell area was regularly assessed. For this estimation, the U-shaped form of each comb was mentally transformed to a virtual rectangular quadrat and the size of this virtual rectangle recorded (length x width). This endpoint allowed to evaluate potential impacts of the test compound on wax gland activity (starting about 13 days after ecdysis). A proper function of the wax glands indicates an appropriate supply of young worker bees. Comb cell production at study termination was compared between control and treatment using a t-test.
- Food consumption:** Pollen and honey was provided as food supply for the honeybees. The amount of pollen and honey consumed was determined by reweighing the respective feeders. The cumulative amount of pollen and honey collected at study termination were compared between control and treatment using a t-test.
- Honey storage behaviour:** The amount of sampled and processed sunflower honey was regularly assessed in two different ways. The weight increase of the small colonies was recorded which reflects mainly the amount of stored honey. The hive weight development at study termination was compared between control and treatment using a t-test. In addition to these weight records, the percentage of comb cells which were filled with honey was also regularly estimated. The percentage values were then converted into an absolute area by taking into account the actual comb cell area at the time of evaluation (see above). The average honey deposition over time per assessment and treatment was compared between control and treatment using a t-test.
- Pollen storage behaviour:** The amount of sampled pollen was regularly assessed. The cumulative amount of pollen collected at study termination was compared between control and treatment using a t-test. The percentage of comb cells which was filled with pollen was regularly estimated. The percentage values were then converted into an absolute area by taking into account the actual comb cell area at the time of evaluation (see above). Pollen deposition over time per assessment and treatment was compared between control and treatment using a t-test.

- Egg laying activity:** The egg laying activity of the queens was assessed by regular inspection of the brood combs. During each inspection, the percentage of comb cells which contained an egg was estimated. The percentage values were then converted into an absolute area by taking into account the actual comb cell area at the time of evaluation (see above). Egg deposition over time per assessment and treatment was compared between control and treatment using a t-test.
- Breeding success:** During each inspection, the percentage of comb cells which contained honeybee larvae or pupae was estimated. The percentage values were then converted into an absolute area by taking into account the actual comb cell area at the time of evaluation (see above). Abundance of larvae and pupae deposition over time per assessment and replicate was compared between control and treatment using a t-test. This endpoint does not only evaluate potential influences of the test compound on the queen health (e.g. egg laying activity, egg fertilization) but also the development of the hypopharyngeal glands of young workerbees. A proper functioning of the hypopharyngeal glands indicates an appropriate supply of young worker bees with pollen which is vital for their nursery activity (between day 4 and 12 after ecdysis).
- Colony strength:** During each inspection, the percentage of comb cell area covered by honeybees was estimated. The percentage values were then converted into an absolute area by taking into account the actual comb cell area at the time of evaluation (see above). This endpoint integrates potential impacts of the test compound on breeding success, longevity and mortality of honeybees. Abundance of adult bees found at the combs over time per assessment and treatment was compared between control and treatment using a t-test.
- Foraging intensity:** Daily except weekends the number of bees foraging during a 5 minute observation period on the honey and pollen feeder were recorded. In addition, the number of honeybees encountered on the tunnel roof was counted. This endpoint may give an indication of possible disorientation or repellent/antifeedant phenomena. Foraging activity was compared between the control and treatment based on the average number of bees found foraging per treatment until study termination.
- Behavioural anomalies:** Whenever observed, behavioural anomalies were recorded with the date and daytime of observation. In particular, honeybees were observed for any of the following symptoms:
- exaggerated motility
 - discoordinated movements (trembling, flight incapability)
 - apathy, lethargic behaviour.

4.0 FILING

All raw data, the study protocol and the original of the report are filed in the Central GLP archive of PF/F, Crop Protection Center 40789 Monheim, FRG. Reserve samples of the test substance are stored in the pertinent archive of that test facility which provided or certified the test substance.

5.0 AMENDMENTS TO THE STUDY PLAN

Amendment 1, dated 2001-06-26:

Setting up of the bees in the tunnels on 2001-06-21

Amendment 2, dated 2001-07-18:

Arrival of the maize pollen from Brazil on 2001-02-09. Details on the harvesting and preparation methods.

Amendment 3, dated 2002-01-23:

Detail on the seed dressing.

Dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt op grond van een wettelijke verplichting tot openbaarmaking.
 Op dit document kunnen rechten van derden rusten, waaronder intellectuele eigendomsrechten en/of auteursrechten.
 Voorts kan dit document onder een regeling omtrent gegevensbescherming vallen, zonder de toestemming van de rechthebbende van dit document kan derhalve verboden zijn en een inbreuk opleveren van de rechten van deze rechthebbende.

This document is not the property of the Ctgb and only provided based on mandatory freedom of information requirements.
 The document may be subject to rights such as intellectual property and copy rights of third parties.
 Furthermore, this document may fall under a regulatory data protection and use of this document or its contents without the permission of the owner of this document may therefore be prohibited and violate the rights of its owner.

Consequently, any publication, distribution, reproduction and/or publishing and any commercial exploitation and use of this document or its contents without the permission of the owner of this document may therefore be prohibited and violate the rights of its owner.

6.0 RESULTS AND DISCUSSION

6.1 Climatic Conditions During the Study

Climatic conditions were recorded in one control tunnel with a thermohygrograph. Records are listed in appendix I. During the study, daily minimum temperatures ranged between 3 and 14°C, maximum temperatures ranged between 20 and 42°C. Minimum air humidity ranged from 26 to 74% and maximum from 84 to 99%. The sky was mostly cloudy during the assessments and wind was mostly slight to modest during the study period.

Additionally data loggers were placed in one control tunnel and recorded temperature, humidity and rainfall from the 2001-06-26 until 2001-08-05. Temperature ranged from 11.0 and 36.9 °C with an average of 21.1 °C. Humidity ranged from 0.2-100.2%* with an average of 65.1%. Rainfall was recorded with 0-13.2 mm and an average of 0.1 mm. The full records are documented in the raw data.

* Due to quick changes in air pressure over a short period of time a relative humidity over 100% may erroneously be recorded for short periods of time.

6.2 Biological Observations on the foraging Honeybees

Mortality

The number of dead bees found in front of the bee hive and at the tunnel edges was recorded and is summarised in appendix II. Table 5 and figure 1 present the total number of the bees per replicate and the average number of bees per treatment found dead in front of the bee hive and at the tunnel edges until removing the hives from the tunnels.

Table 5: Mortality of the bees in front of the bee hive and at the tunnel edges

Treatment	1a	1b	1c	3a	3b	3c
Total number of dead bees found in front of the bee hive per replicate	1	7	0	5	1	3
Average number of dead bees found in front of the bee hive per treatment group	0.67			3.00		
Total number of dead bees found at the tunnel edges per replicate	28	31	25	50	34	50
Average number of dead bees found at the tunnel edges per treatment group	28.00			44.67		

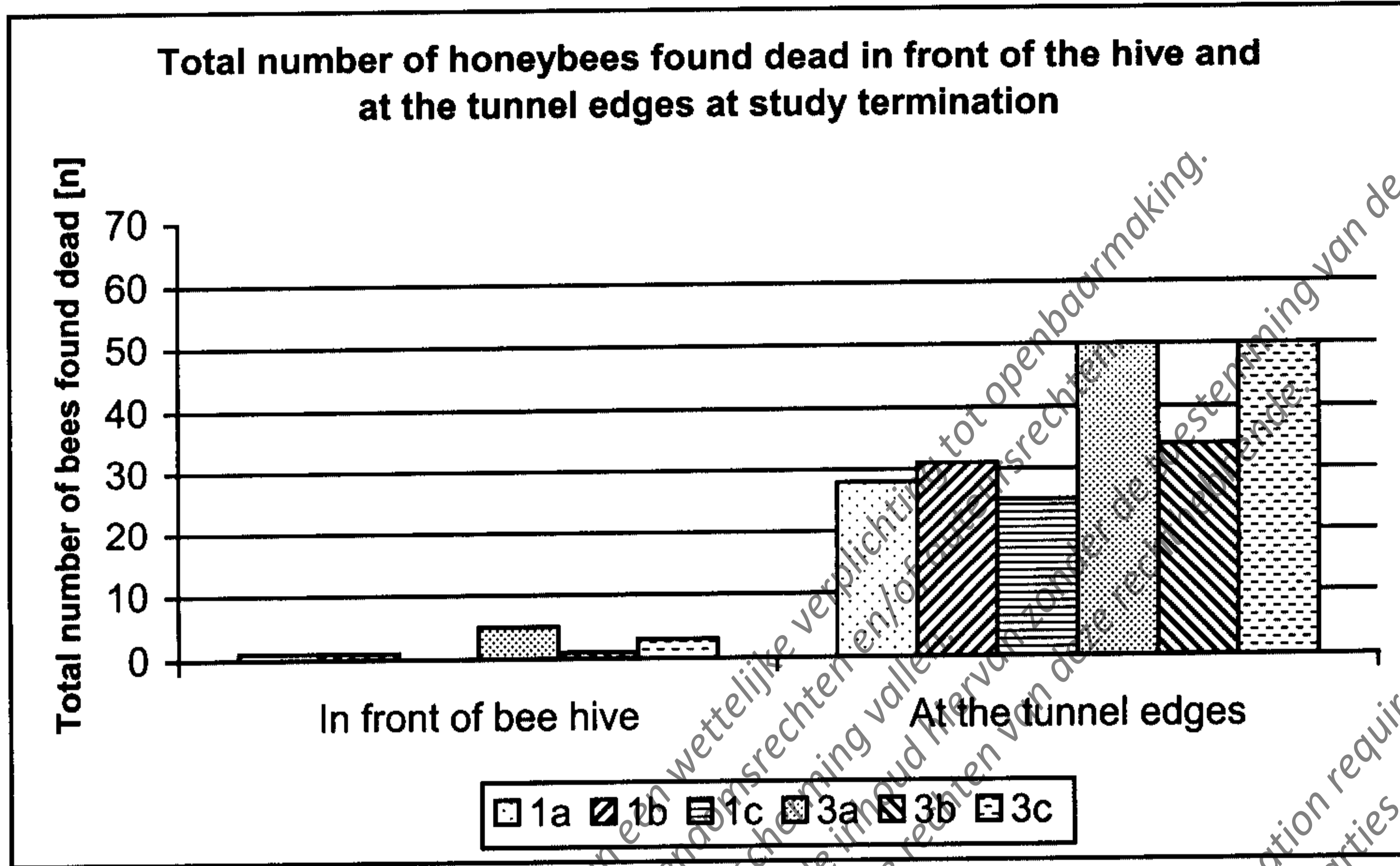


Figure 1: Mortality of the bees in front of the bee hive and at the tunnel edges

Mortality in front of the bee hives was comparably low in control and treatment. The number of dead bees found at the tunnel edges, although higher in treatment than in control, was also comparable in both groups.

The slightly higher mortality in the treatment compared with the control lies within the range of variability which typically occurs in studies of this design, and is therefore not considered to be treatment related.

Dit document is geen eigendom van het Ctgb en wordt beschermd op grond van de wettelijke verplichting tot openbaarmaking.
 Op dit document kunnen rechten van derden rusten, waaronder intellectuele eigendomsrechten en/of andere rechten van derden.
 Voorts kan dit document onder een auteursrecht of andere rechten van derden vallen.
 Publicatie, verspreiding, vermenigvuldiging, commerciële exploitatie en gebruik van dit document kan derhalve verboden zijn.
 This document is not the property of the Ctgb and only provided based on information requirements of third parties.
 The document may be subject to rights such as intellectual property and copy protection.
 Consequently, any publication, distribution and/or publishing and any commercial exploitation may therefore be prohibited.

Comb production

The comb cell production was recorded throughout the study period and is summarised in appendix III. The size of the comb area up to study day 52 are presented in table 6 and figure 2.

Table 6: Area of comb cell production in cm²

Study day	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
8	276	289	312	285	316	408
14	492	504	456	444	492	516
21	264	534	492	528	567	588
29	572	556	492	540	567	588
37	572	556	492	540	567	588
45	648	672	584	600	639	713
52	768	708	675	692	651	768

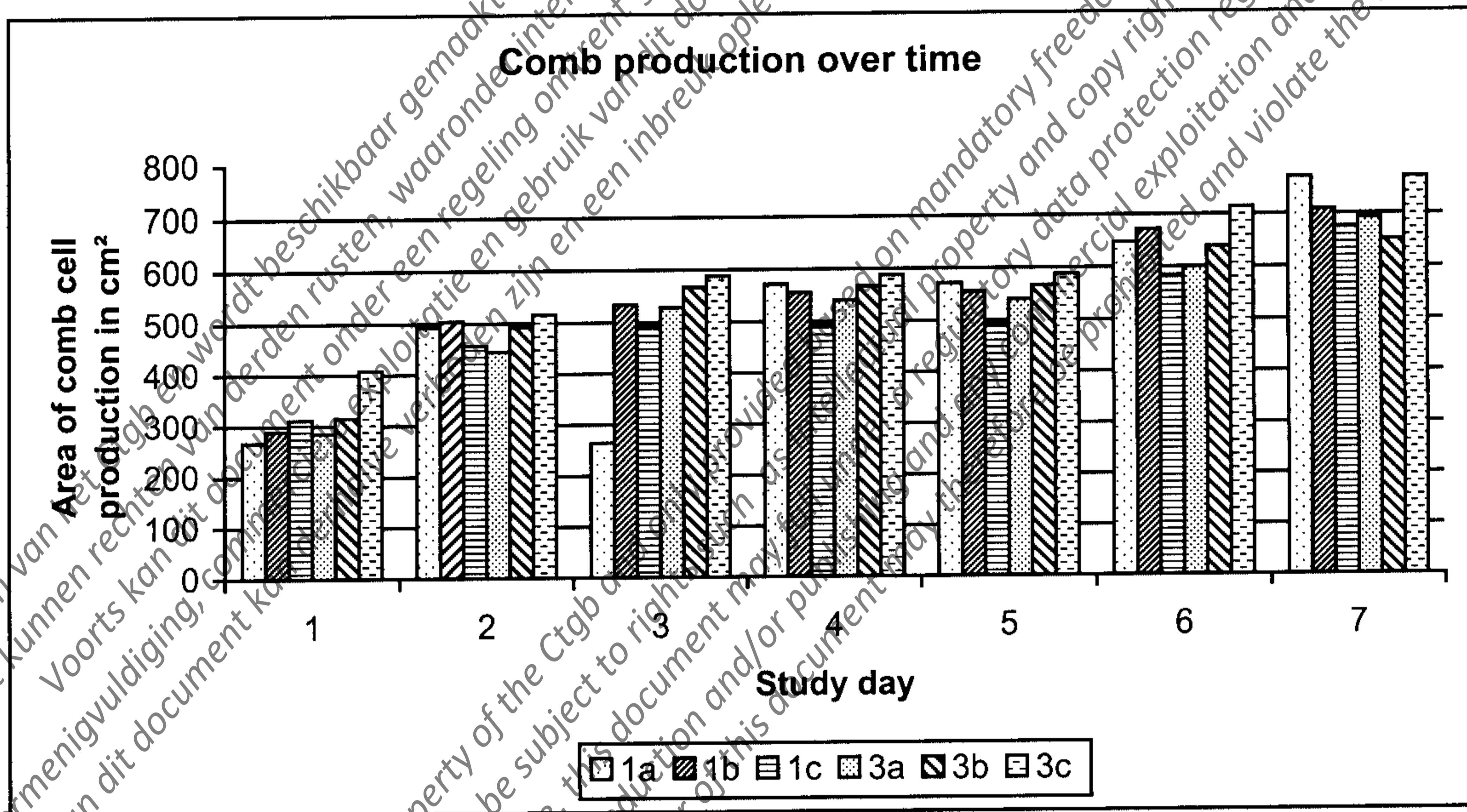


Figure 2: Area of comb cell production in cm²

All colonies started immediately with the production of new comb cells. No significant differences in comb cell production were found until study day 52 ($t=-0.478$, $p=0.641$). Obviously, the test item did not affect this endpoint.

Honeybee Semifield Test Imidacloprid

Honey consumption

The quantity of honey collected by the foraging bees was recorded throughout the study period and is summarised in appendix IV and illustrated in table 7 and figure 3. Columns in figure 3 show the cumulative quantity of honey collected by the foraging honeybees until study day 45.

Table 7: Honey consumption

Study day	Cumulative honey consumption [g]	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
45	per replicate	701.86	694.15	677.42	621.00	661.29	667.58
45	Average per treatment group	691.14			649.96		

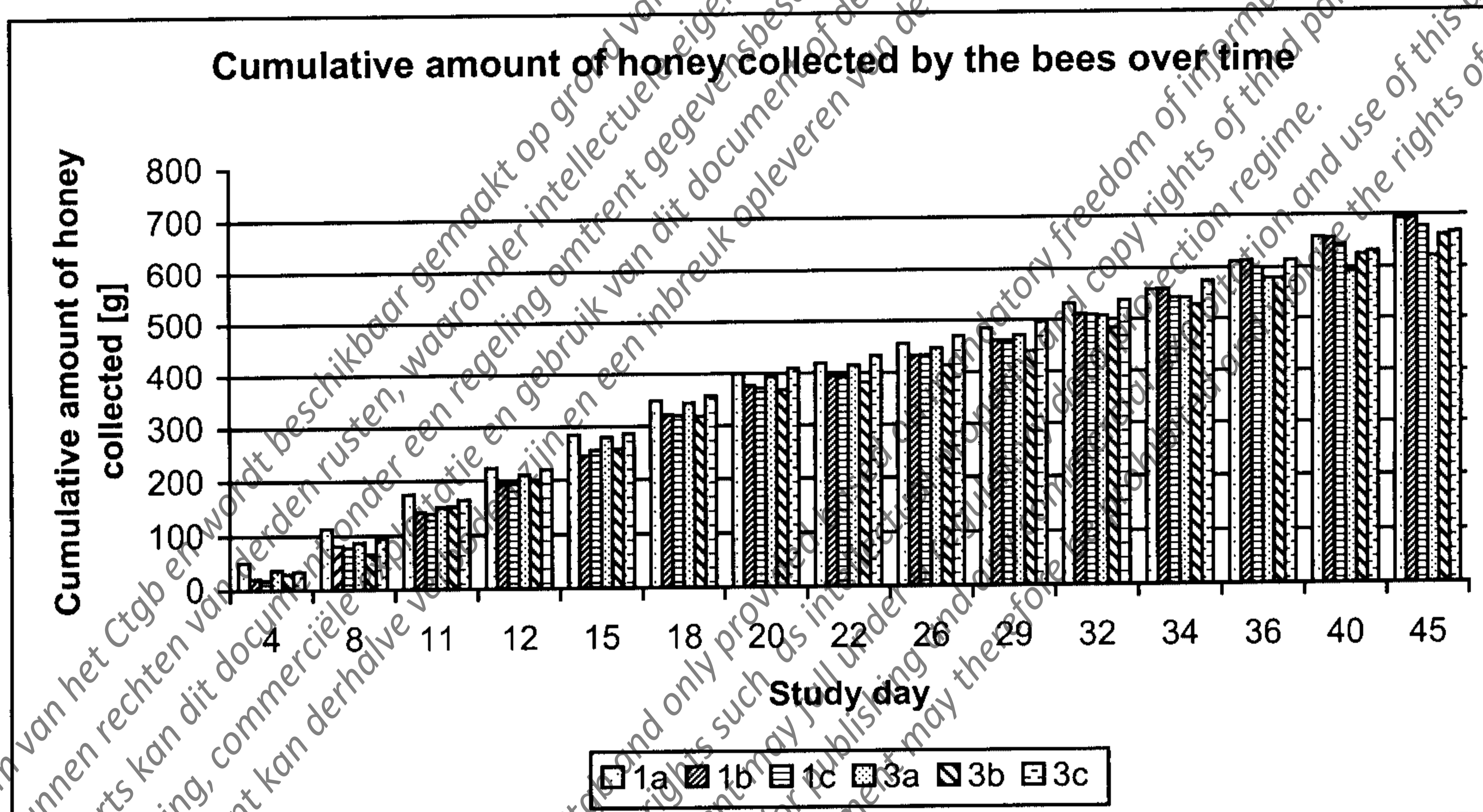


Figure 3: Honey consumption

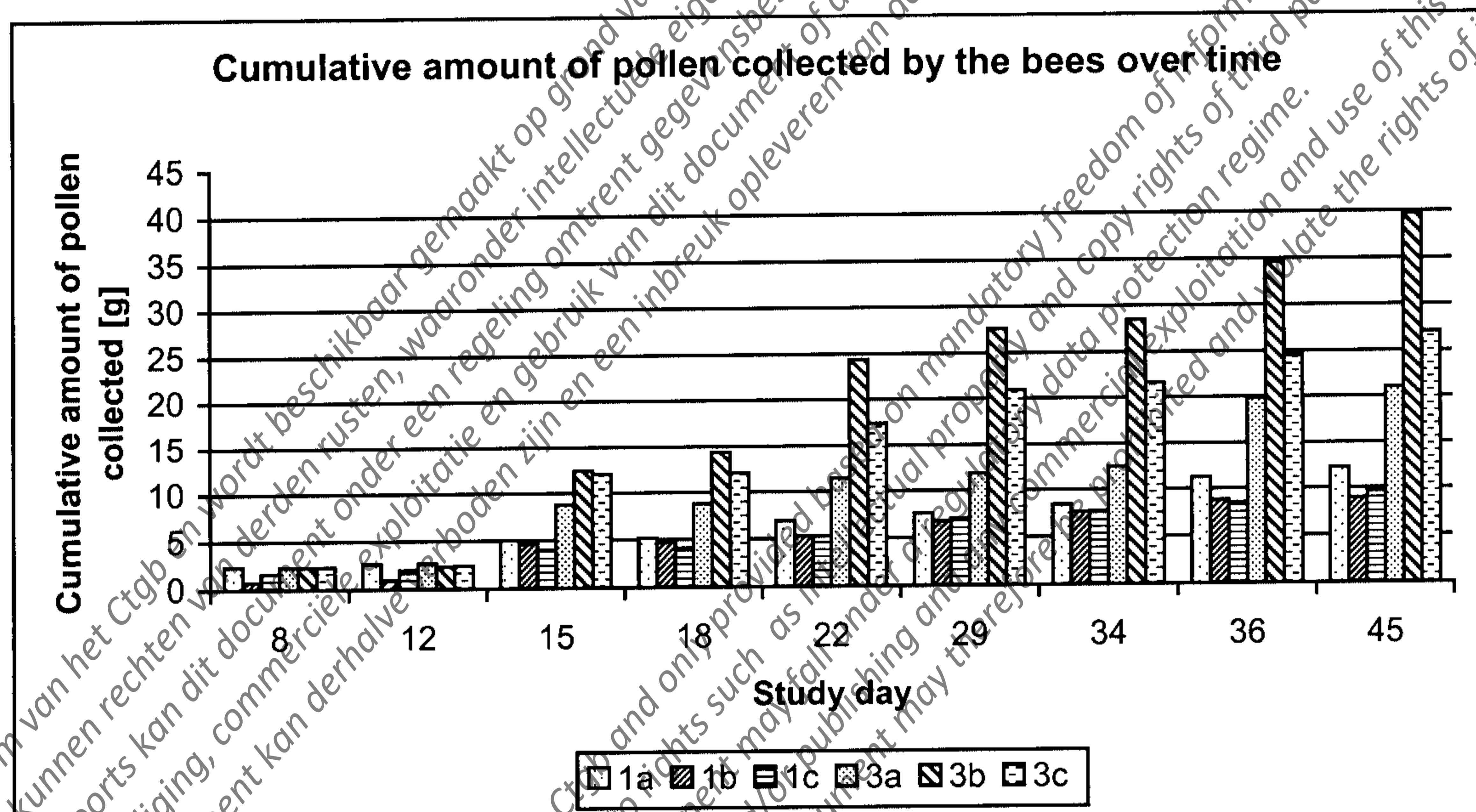
At study termination the overall amount of honey collected was largely the same in all control and treatment groups. No significant differences in honey consumption were found until study day 45 ($t=2.530$, $p=0.065$), a treatment effect was not found.

Pollen consumption

The quantity of pollen collected by the foraging bees was recorded throughout the study period and is summarised in appendix IV and illustrated in table 8 and figure 4. Columns show the cumulative quantity of pollen collected by the foraging honeybees until study day 45.

Table 8: Pollen consumption

Study day	Cumulative pollen consumption [g]	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
45	per replicate	12.20	8.89	9.62	27.01	39.75	27.06
45	Average per treatment group	10.24			29.27		

*Figure 4. Pollen consumption*

The average overall amount of pollen collected per treatment until study day 45 was significantly higher in the Imidacloprid treatment than in the control ($t=-3.391$, $p=0.027$). This can be explained by the granulation of the pollen: The treatment pollen was more pulverulent than the control pollen and allowed an easier transport by the bees. The differences in the granulation of the pollen occurred due to the harvesting conditions. The treatment pollen was harvested during wet and the control pollen during dry weather.

Honey stores

The quantity of honey stores found in the combs was recorded throughout the study period and is summarised in appendix V and table 9 and illustrated in figure 5. Columns show the quantity of honey collected by the foraging honeybees over time.

Table 9: Comb area with honey stores in cm² comb area

Study day	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
8	46	46	30	32	67	50
14	204	210	154	177	163	167
21	299	281	237	236	258	271
29	287	264	239	258	225	257
37	325	309	276	296	323	306
45	229	274	198	200	246	306
52	194	234	116	133	172	226

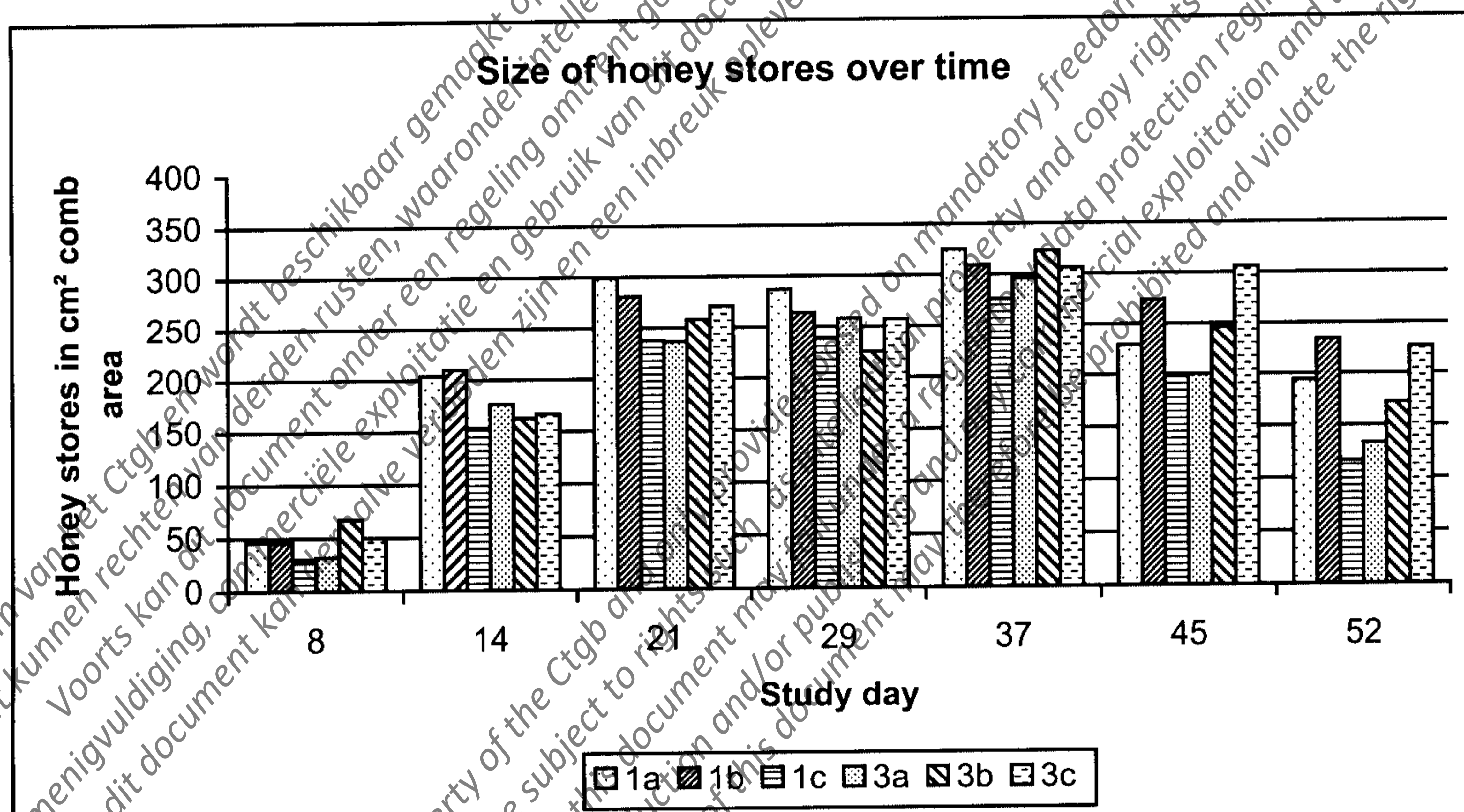


Figure 5: Comb area with honey stores in cm² comb area

The amount of the honey stores fluctuated considerably over time in all control and treatment groups. These fluctuations must be considered to be caused by the food consumption associated with breeding activity of the bee colonies.

In the development of the honey stores over time, no statistically significant differences between control and treatment were found (t=0.086, p=0.933). Individual variation was very high during all assessments, and differences were obviously determined by this natural variation. No signs for a treatment related effect were found.

Honeybee Semifield Test Imidacloprid

Pollen stores

The quantity of pollen stores found in the combs was recorded throughout the study period and is summarised in appendix VI and table 10 and illustrated in figure 6. Columns show the quantity of pollen collected by the foraging honeybees over time.

Table 10: Comb area with pollen stores in cm² comb area

Study day	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
8	0	0	1	1	17	2
14	5	0	14	15	23	0
21	0	4	0	4	11	5
29	0	0	0	0	0	0
37	0	0	0	0	18	0
45	46	12	9	8	22	5
52	41	13	18	17	25	27

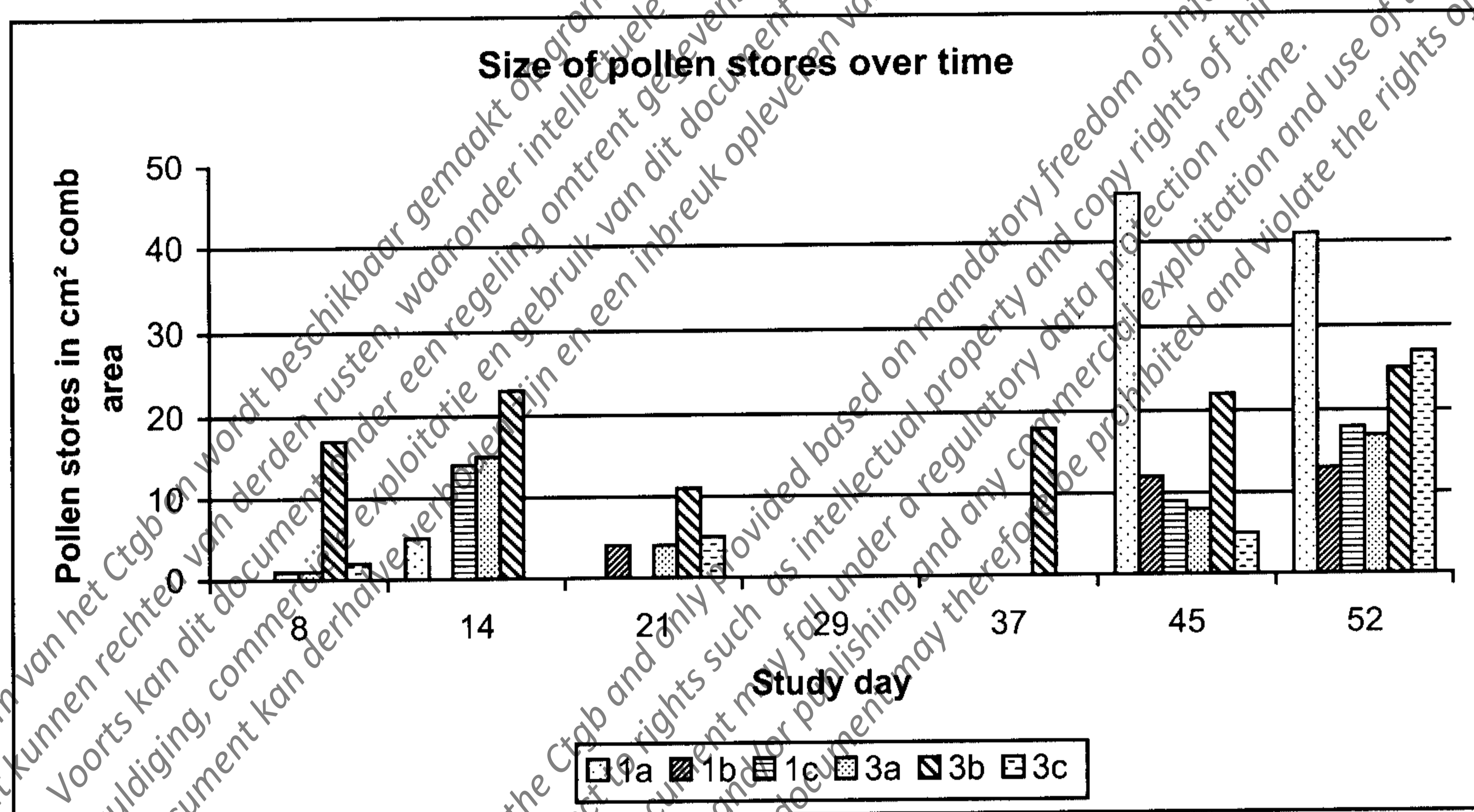


Figure 6: Comb area with pollen stores in cm² comb area

The amount of the pollen stores fluctuated considerably over time in all control and treatment groups. These fluctuations must be considered to be caused by the food consumption associated with breeding activity of the bee colonies. The amount of pollen stored was very variable over the assessments but there were no significant differences between control and treatment ($t=-0.360$, $p=0.725$).

Likewise, it is evident from the breeding performance of all colonies tested in this study that honeybees of all treatment groups collected and fed sufficient pollen to allow a strong population increase. A treatment-related effect was not found in this endpoint.

Honeybee Semifield Test Imidacloprid

Weight development of the bee hives

The hive weight was recorded throughout the study and is summarised in appendix VII and presented in table 11 and figure 7.

Table 11: Weight development of the bee hives

Study day	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
43	1180	1225	1160	1120	1125	1125
Total hive weight gain [g]	240	265	210	165	215	200
Weight gain per replicate [%]	25.53	27.60	22.17	17.28	23.63	21.62
Average weight gain per treatment [%]	25.08			20.84		

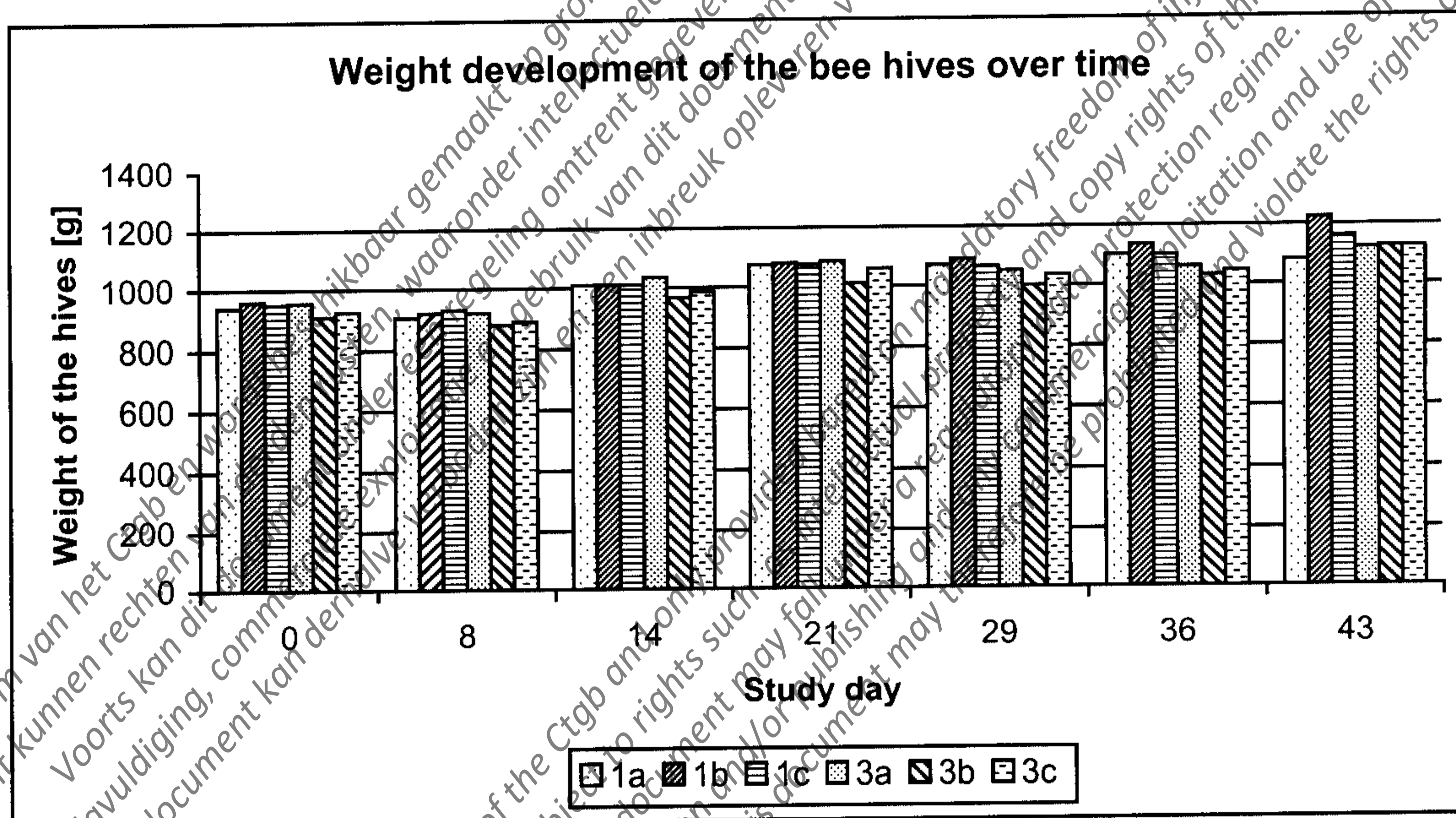


Figure 7: Weight development of the bee hives

All hive colonies increased in weight during their confinement on the study plots. The increase of the hive weight was comparable between the replicates tested, and no treatment-related effect is indicated. There were no significant differences between control and treatment until study day 43 ($t=1.720$, $p=0.161$). Obviously, this endpoint was not influenced by the test item.

Foraging activity

Foraging activity was assessed by counting the number of honeybees observed at the tunnel roofs, the pollen and the honey feeders and is summarised in the appendix XII. Table 10 and figure 8 present the average number of honeybees per replicate and assessment and per treatment and assessment found at the tunnel roofs and the feeders. Columns in figure 8 give the average number of foraging honeybees recorded per assessment at the tunnel roofs, the pollen feeder or the honey feeders.

Table 12: Foraging activity of the honeybees

	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
Average number of bees per replicate and assessment at the tunnel roof	2.82	3.50	2.25	29.3	3.25	2.89
Average number of bees per treatment and assessment at the tunnel roof	2.86			3.02		
Average number of bees per replicate and assessment at the pollen feeder	0.71	0.71	0.71	0.79	1.07	0.61
Average number of bees per treatment and assessment at the pollen feeder	0.71			0.82		
Average number of bees per replicate and assessment at the honey feeder	7.29	8.11	7.71	7.29	8.21	7.86
Average number of bees per treatment and assessment at the honey feeder	7.70			7.79		

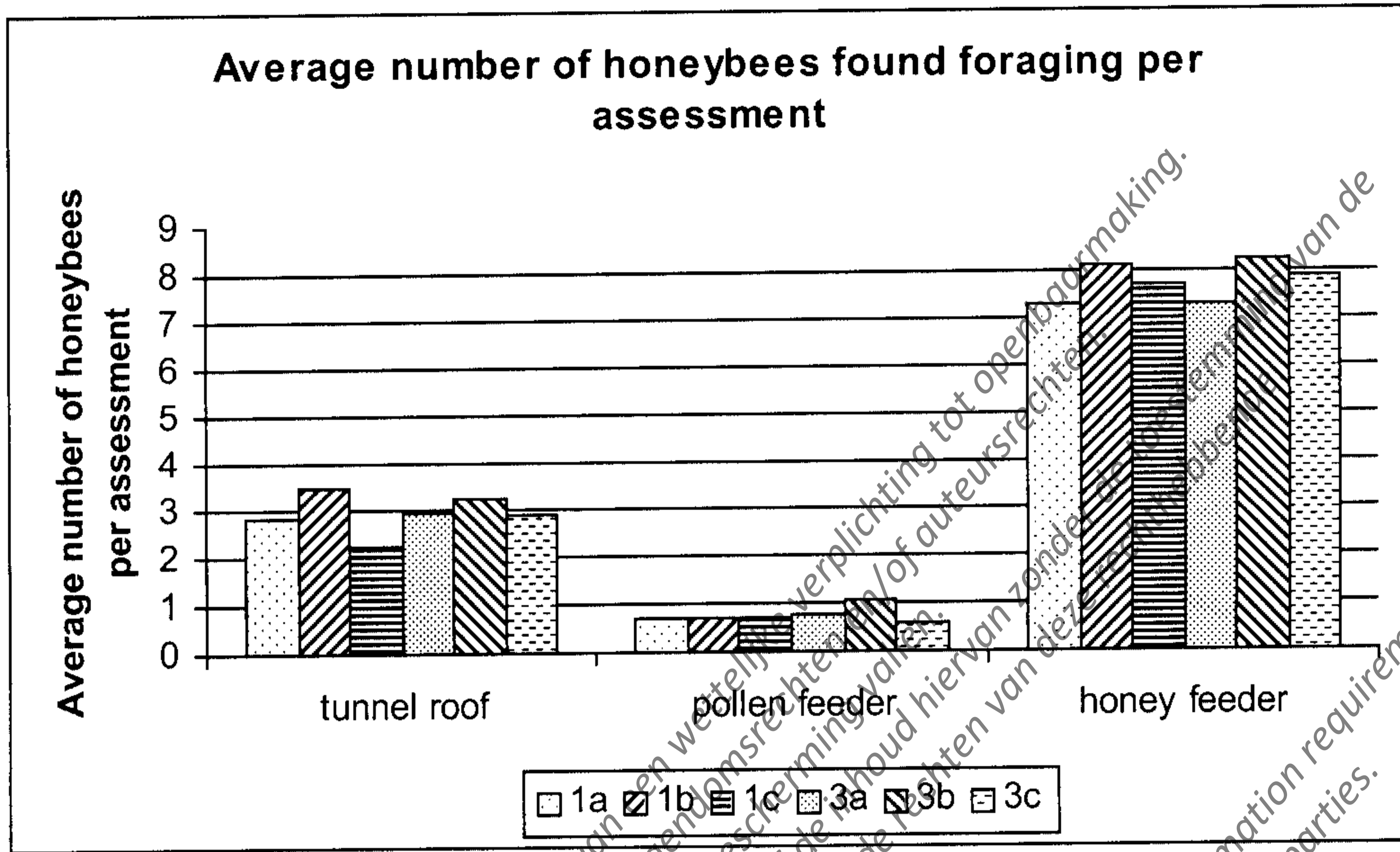


Figure 8: Foraging activity of the honeybees

The average number of honeybees per treatment and assessment observed at the tunnel roofs, the pollen feeder and the honey feeders was comparable between treatment and control, which indicates the absence of any treatment effect in this endpoint.

Dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt op grond van een wettelijk verplichting tot openbaarmaking.
 Op dit document kunnen rechten van derden rusten, waaronder intellectuele eigendomsrechten/ of auteursrechten.
 Voorts kan dit document onder een regeling van de wet op de auteursrechten vallen.
 Publicatie, verspreiding, vermenigvuldiging, commerciële exploitatie en gebruik van de inhoud hiervan zonder toestemming van de rechthebbende van dit document kan derhalve verboden zijn en een inbreuk opleveren van de rechten van de rechthebbende van de
 This document is not the property of the Ctgb and only provided based on mandatory freedom of information requirements.
 The document may be subject to rights such as intellectual property and copy rights of third parties.
 Consequently, any publication, distribution, reproduction and/or publishing and any commercial exploitation and use of this document or its contents without the permission of the owner of this document may therefore be prohibited and constitute an infringement of the rights of its owner.

6.3 Population Development and Breeding Performance

Egg laying activity

Egg laying activity which was assessed and recorded throughout the study period is summarised in appendix VIII and presented in table 13 and illustrated in figure 9. Columns show the area of combs with cells containing eggs

Table 13: Comb area with cells containing eggs in cm² comb area

Study day	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
8	165	165	149	119	124	186
14	115	143	117	91	170	149
21	72	90	93	69	88	63
29	45	59	47	50	61	72
37	68	38	56	38	51	53
45	165	194	140	96	198	261
52	177	98	130	182	125	181

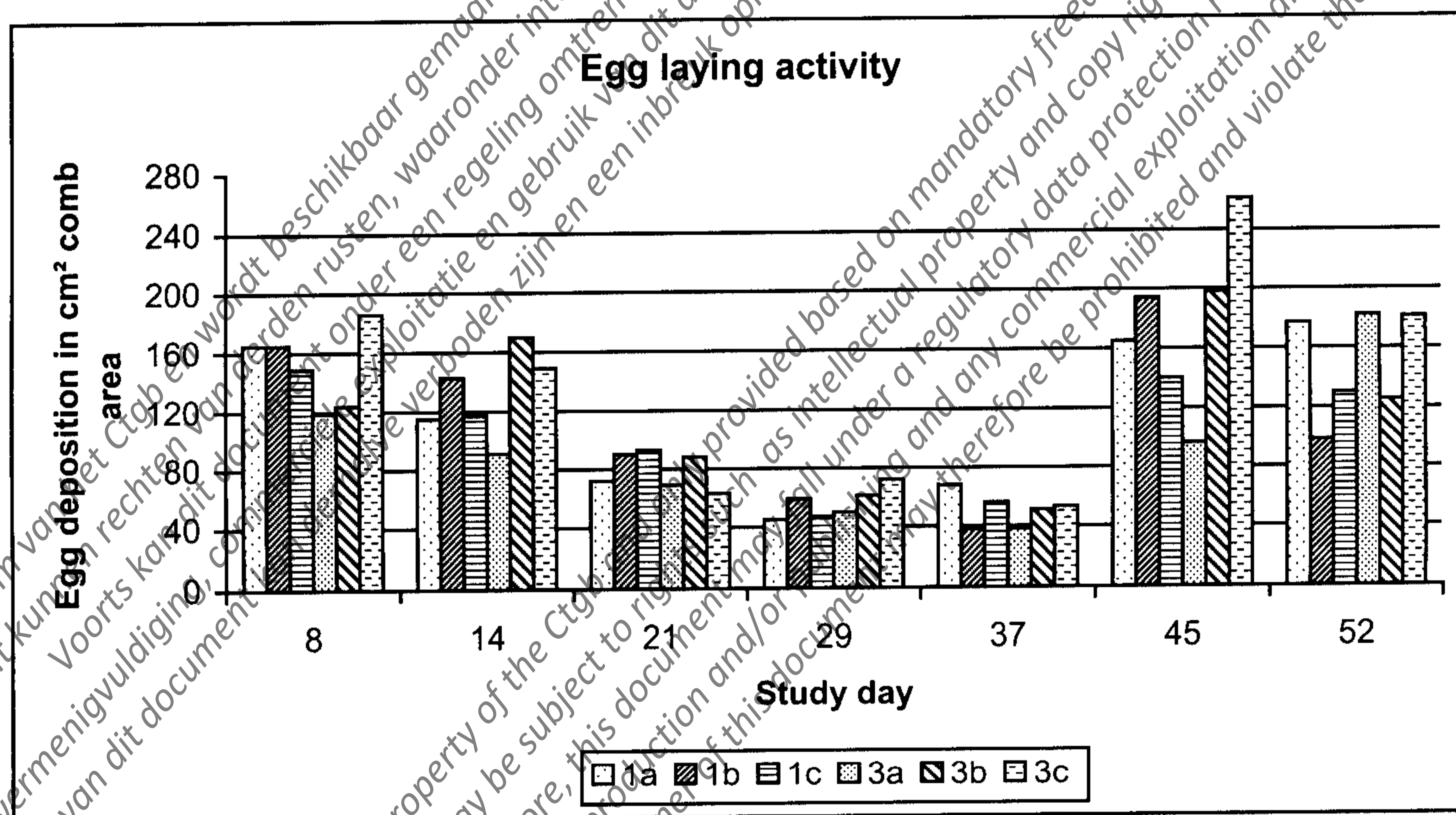


Figure 9: Comb area with cells containing eggs in cm² comb area

Egg-laying activity was variable in control and treatment groups but no significant differences were observed between control and treatment (t=-0.176, p=0.863). On the different assessment dates the abundance of honeybee eggs was alternately higher in the control and in the treatment. No evidence for a treatment effect was found.

Abundance of honeybee larvae

Abundance of honeybee larvae was assessed and recorded throughout the study period and is summarised in appendix IX and presented in table 14 and illustrated in figure 10. Columns show the area of combs with cells containing larvae.

Table 14: Comb area with cells containing larvae in cm² comb area

Study day	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
8	0	8	27	3	0	34
14	34	8	22	12	52	24
21	14	17	24	29	56	29
29	9	8	14	8	17	14
37	9	8	9	8	13	10
45	59	72	62	34	62	64
52	92	70	79	99	67	120

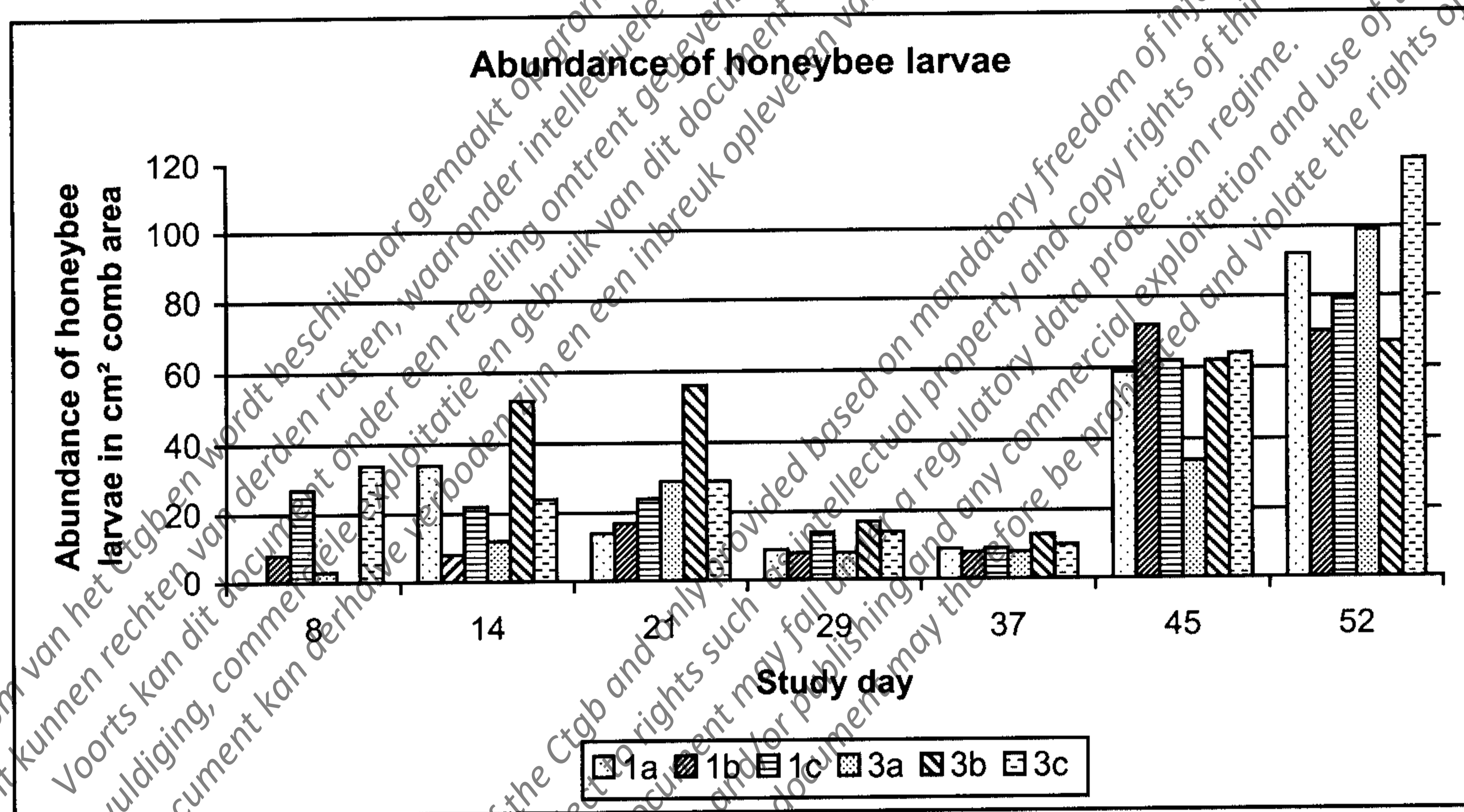


Figure 10: Comb area with cells containing larvae in cm² comb area

Larval abundance was variable in control and treatment groups but no significant difference between control and treatment could be observed (t=-0.328, p=0.749). On the different assessment dates the abundance of honeybee larvae was alternately higher in the control or in the treatments. The observed differences have to be attributed to natural variability. No treatment-related differences were found in this testing endpoint.

Abundance of honeybee pupae

Abundance of honeybee pupae was assessed and recorded throughout the study period and is summarised in appendix X and presented in table 15 and illustrated in figure 11. Columns show the area of combs with cells containing pupae.

Table 15: Comb area with cells containing pupae in cm² comb area

Study day	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
8	0	0	0	0	0	0
14	8	13	19	16	0	29
21	36	29	38	38	51	76
29	32	17	29	25	68	38
37	9	8	9	8	9	10
45	9	5	14	8	43	14
52	113	92	149	86	110	125

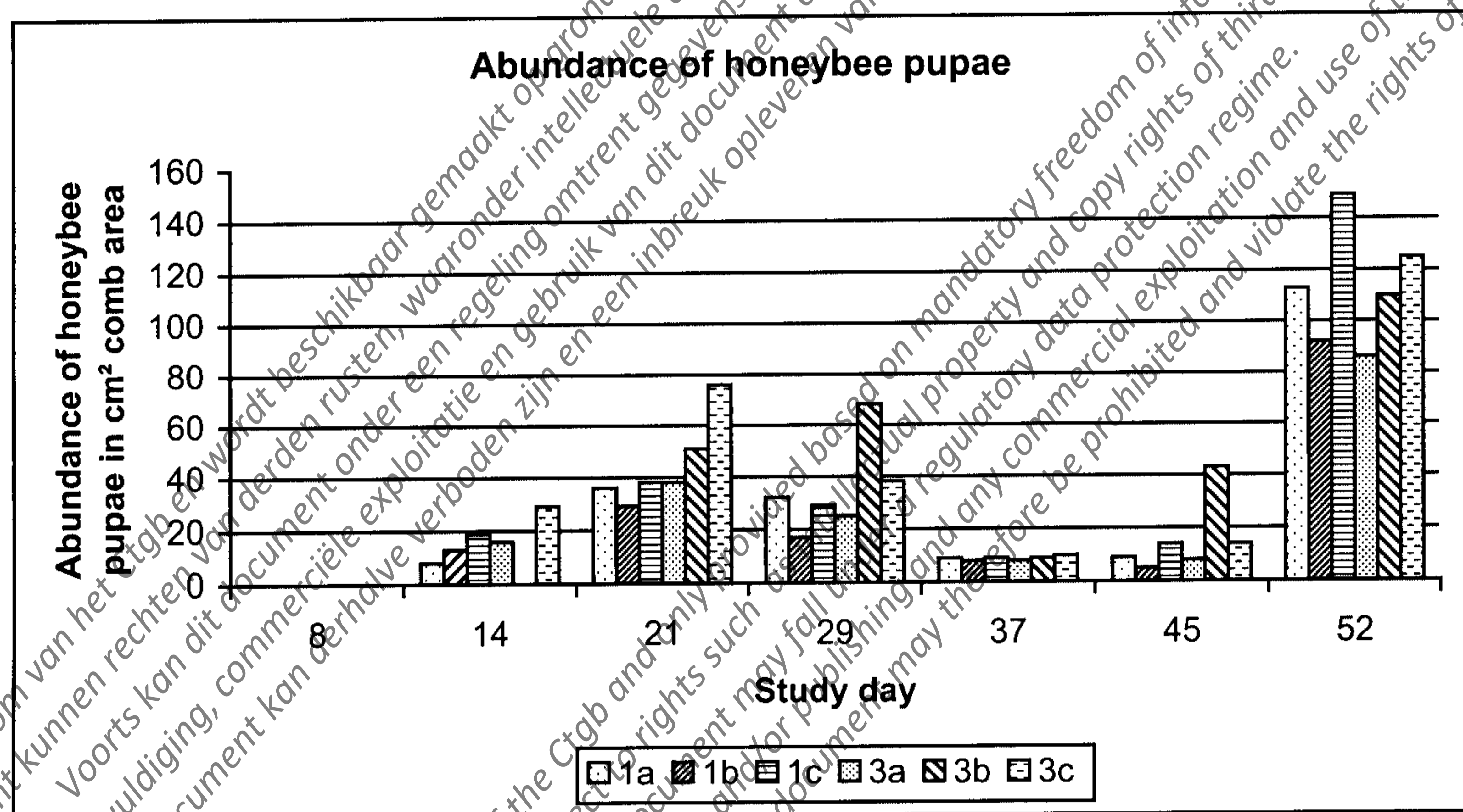


Figure 11: Comb area with cells containing pupae in cm² comb area

The abundance of honeybee pupae was variable in control and treatment groups but no significant differences between control and treatment could be observed ($t=0.288$, $p=0.778$). The observed differences have to be attributed to natural variability, no treatment effect could be shown to exist.

Population development

The proportions of comb area covered by adult honeybees throughout the study was assessed during the evaluation checks and is presented in appendix XI and in table 16 and illustrated in figure 12. Columns in figure 12 show the population development over time during the study.

Table 16: Comb area covered with adult honeybees during assessments in cm²

Study day	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
8	151	138	223	175	141	190
14	212	224	196	178	199	292
21	259	233	266	224	231	261
29	205	256	233	202	178	231
37	151	140	163	157	148	139
45	315	295	242	238	264	375
52	266	183	183	265	222	260

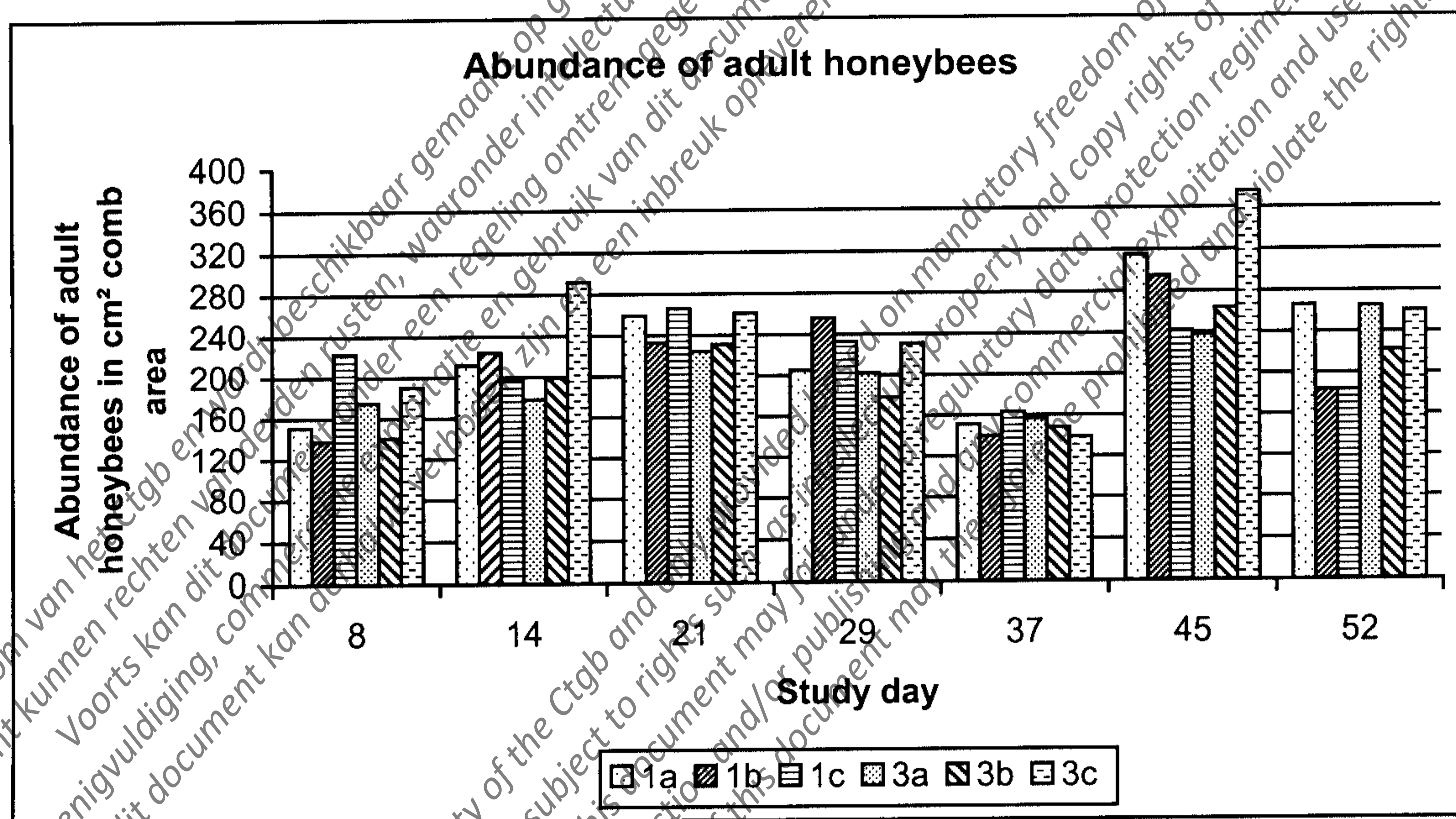


Figure 12: Comb area covered with adult honeybees during assessments in cm²

The bee population showed the same trend for all treatment and control groups to increase and decrease over time in no regular pattern. There were no significant differences observed between control and treatment ($t=-0.068, p=0.947$). No evidences for a treatment effect were found.

Honeybee Semifield Test Imidacloprid

6.4 Summary of the Analytical Findings of grown residues of Imidacloprid FS 600 in pollen

Sample Material	Treatment	Hydroxy-Imidacloprid [mg/kg] *	Olefin-Imidacloprid [mg/kg] *	Imidacloprid [mg/kg] *
Maize Pollen, No. K-1A	Control	n.d./n.d.	n.d./n.d.	n.d./n.d.
Maize Pollen, No. K-1B	Control	n.d./n.d.	n.d./n.d.	n.d./n.d.
Maize Pollen, No. K-2A	Control	n.d./n.d.	n.d./n.d.	n.d./n.d.
Maize Pollen, No. K-2B	Control	n.d./n.d.	n.d./n.d.	n.d./n.d.
Maize Pollen, No. B-01	NTN 33893 FS 600	n.d./n.d.	n.d./n.d.	n.d./n.d.
Maize Pollen, No. B-02	NTN 33893 FS 600	n.d./n.d.	n.d./n.d.	n.d./n.d.

Limit of quantitation: 0.005 mg/kg for Imidacloprid and Hydroxymetabolite, 0.01 mg/kg for Olefinmetabolite

<0.005 and <0.010: Residues below the limit of quantation (<LOQ)

Limit of detection: 0.0015 mg/kg for Imidacloprid and Hydroxymetabolite, 0.003 mg/kg for Olefinmetabolite

n.d.: Residues below the limit of detection

7.0 CONCLUSION

The results of the study show that there is no risk to honeybees by foraging on and consumption of maize pollen of plants originating from seeds dressed with Imidacloprid FS 600 at the rate of 1 g a.i./1000 kernels.

APPENDICES

APPENDIX I: Climatic Conditions as Recorded During Evaluation Dates

Records were made within the study tunnels using a thermohygrograph.

Study day	Minimum Temperature [° C]	Maximum Temperature [° C]	Minimum Air Humidity [%]	Maximum Air Humidity [%]
1	3	24	55	99
2	6	30	42	99
3	7	31	39	96
4	11	36	39	92
5	8	38	30	95
6	12	35	49	95
7	11	31	46	95
8	12	31	50	94
9	10	34	49	95
10	9	29	49	94
11	8	28	70	96
12	7	30	49	96
13	8	31	45	95
14	11	35	40	95
15	9	35	39	95
16	13	26	54	98
17	11	20	65	96
18	10	29	40	90
19	8	29	35	96
20	7	25	38	97
21	6	25	37	95
22	6	27	34	96
23	8	22	74	96
24	6	27	38	95
25	8	20	54	96
26	7	31	30	96
27	10	30	36	96
28	9	28	40	95
29	7	22	60	96
30	6	26	41	95
31	12	35	35	93
32	12	39	40	95
33	12	36	35	95
34	11	38	34	96
35	12	38	33	96
36	13	42	26	95
37	14	37	35	96
38	14	36	28	96
39	13	38	35	85
40	13	39	32	94
41	10	35	30	95
42	9	37	31	95
44	13	20	40	93
45	10	21	39	84

Honeybee Semifield Test Imidacloprid

APPENDIX II: Mortality in Control and Treatment

The table gives the number of honeybees (worker bees and drones) which were found dead during the study in front of the bee hives.

Days after first exposure to treated substrate	Number of dead honeybees found in front of the bee hives					
	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
1	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
2	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
3	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
4	1	0	0	1	1	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
11	0	0	0	0	0	0
12	0	0	0	0	0	0
13	0	0	0	0	0	0
14	0	0	0	0	0	0
15	0	0	0	0	0	0
18	0	0	0	0	0	0
19	0	0	0	0	0	0
20	0	0	0	0	0	0
21	0	0	0	0	0	0
22	0	0	0	0	0	0
25	0	0	0	0	0	0
26	0	0	0	0	0	0
27	0	0	0	1	0	0
28	0	0	0	0	0	0
32	0	0	0	0	0	0
33	0	0	0	0	0	2
34	0	0	0	1	0	0
35	0	0	0	0	0	0
36	0	0	0	0	0	0
39	0	0	0	0	0	0
40	0	0	0	0	0	0
41	0	0	0	2	0	0
42	0	0	0	0	0	0
Total	1	1	0	5	1	3

n.r. – not reported

Dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt op grond van een wettelijke verplichting tot openbaarmaking. Op dit document kunnen rechten van derden rusten, waaronder intellectuele eigendomsrechten en/of auteursrechten. Publicatie, verspreiding, vermenigvuldiging, commercieel gebruik van dit document of de inhoud hiervan zonder de toestemming van de rechthebbende van dit document kan derhalve verboden zijn en een inbreuk opleveren van de rechten van de rechthebbende. This document is not the property of the Ctgb and only provided based on mandatory freedom of information requirements. Consequently, any publication, distribution, reproduction and/or publishing and any commercial exploitation and use of this document or its contents without the permission of the owner of this document may therefore be prohibited and violate the rights of its owner.

Honeybee Semifield Test Imidacloprid

Contd'Appendix II: Mortality in Control and Treatment

The table presents the number of honeybees (worker bees and drones) which were found dead during the study at the tunnel edges.

Days after first exposure to treated substrate	Number of dead honeybees found at the tunnel edges					
	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
1	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
2	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
3	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
4	4	0	0	7	6	0
5	0	2	0	2	0	2
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
11	0	2	0	0	0	0
12	0	0	0	0	0	0
13	0	0	0	0	1	0
14	0	0	0	0	0	0
15	0	0	2	0	0	1
18	0	0	0	0	0	0
19	0	0	0	1	0	0
20	0	1	1	1	0	0
21	1	1	0	0	1	2
22	2	0	1	2	2	1
25	3	0	1	8	2	4
26	4	0	1	5	1	2
27	0	3	3	1	1	4
28	1	1	1	1	0	8
32	3	8	6	8	5	11
33	0	4	0	4	4	2
34	0	0	0	3	0	2
35	2	2	4	1	3	0
36	2	3	1	1	4	2
39	3	0	0	1	3	2
40	0	0	1	1	0	1
41	3	0	1	2	1	2
42	0	4	2	1	0	4
Total	28	31	25	50	34	50

n.r. – not reported

Honeybee Semifield Test Imidacloprid

APPENDIX III: Comb Production in Control and Treatment

The table gives the production of comb cells on four comb matrices over time, i.e. cumulative values. The newly produced comb area was not rectangular. Instead, it was added on to the rectangularly shaped comb matrix in the form of a half circle. The area of the irregularly shaped comb was estimated by extrapolation of this geometric form into a rectangular form. The values in parenthesis give the one-sided mean vertical and the mean horizontal extension of this extrapolated quadrat for each of the four comb matrices. Since comb cells are produced simultaneously on both sides, the total area calculated had to be multiplied by factor 2.

The "total" values give the total comb cell area of 4 combs in cm².

Days after first exposure to treated substrate/ comb area	Increase in comb area [cm ²]					
	Control	Control	Control	Treatment	Treatment	Treatment
	1a	1b	1c	3a	3b	3c
8	168 (12x7)	84 (7x6)	180 (12x7.5)	144 (12x6)	60 (6x5)	192 (12x8)
	108 (9x6)	117 (9x6.5)	132 (12x5.5)	121 (11x5.5)	120 (10x6)	156 (12x6.5)
	0 (0x0)	88 (8x5.5)	0 (0x0)	20 (5x2)	96 (8x6)	60 (10x3)
	0 (0x0)	0 (0x0)	0 (0x0)	0 (0x0)	40 (5x4)	0 (0x0)
	Total: 276	Total: 289	Total: 312	Total: 285	Total: 316	Total: 408
14	192 (12x8)	120 (10x6)	192 (12x8)	168 (12x7)	80 (8x5)	192 (12x8)
	168 (12x7)	168 (12x7)	168 (12x7)	156 (12x6.5)	168 (12x7)	192 (12x8)
	132 (12x5.5)	156 (12x6.5)	96 (12x4)	120 (12x5)	156 (12x6.5)	132 (12x5.5)
	0 (0x0)	60 (10x3)	0 (0x0)	0 (0x0)	88 (8x5.5)	0 (0x0)
	Total: 492	Total: 504	Total: 456	Total: 444	Total: 492	Total: 516
21	192 (12x8)	144 (12x6)	192 (12x8)	168 (12x7)	99 (9x5.5)	192 (12x8)
	180 (12x7.5)	168 (12x7)	180 (12x7.5)	168 (12x7)	180 (12x7.5)	192 (12x8)
	144 (12x6)	156 (12x6.5)	120 (12x5)	144 (12x6)	168 (12x7)	156 (12x6.5)
	48 (8x3)	66 (11x3)	0 (0x0)	48 (8x3)	120 (10x6)	48 (8x3)
	Total: 564	Total: 534	Total: 492	Total: 528	Total: 567	Total: 588
29	192 (12x8)	144 (12x6)	192 (12x8)	168 (12x7)	99 (9x5.5)	192 (12x8)
	180 (12x7.5)	168 (12x7)	180 (12x7.5)	168 (12x7)	180 (12x7.5)	192 (12x8)
	144 (12x6)	156 (12x6.5)	120 (12x5)	156 (12x6.5)	168 (12x7)	156 (12x6.5)
	56 (8x3.5)	88 (11x4)	0 (0x0)	48 (8x3)	120 (10x6)	48 (8x3)
	Total: 572	Total: 556	Total: 492	Total: 540	Total: 567	Total: 588
37	192 (12x8)	144 (12x6)	192 (12x8)	168 (12x7)	99 (9x5.5)	192 (12x8)
	180 (12x7.5)	168 (12x7)	180 (12x7.5)	168 (12x7)	180 (12x7.5)	192 (12x8)
	144 (12x6)	156 (12x6.5)	120 (12x5)	156 (12x6.5)	168 (12x7)	156 (12x6.5)
	56 (8x3.5)	88 (11x4)	0 (0x0)	48 (8x3)	120 (10x6)	48 (8x3)
	Total: 572	Total: 556	Total: 492	Total: 540	Total: 567	Total: 588
45	192 (12x8)	156 (12x6.5)	192 (12x8)	168 (12x7)	99 (9x5.5)	192 (12x8)
	180 (12x7.5)	180 (12x7.5)	180 (12x7.5)	168 (12x7)	180 (12x7.5)	192 (12x8)
	144 (12x6)	168 (12x7)	132 (12x5.5)	156 (12x6.5)	192 (12x8)	168 (12x7)
	132 (11x6)	168 (12x7)	80 (8x5)	108 (9x6)	168 (12x7)	161 (11.5x7)
	Total: 648	Total: 672	Total: 584	Total: 600	Total: 639	Total: 713
52	192 (12x8)	168 (12x7)	192 (12x8)	168 (12x7)	99 (9x5.5)	204 (12x8.5)
	204 (12x8.5)	180 (12x7.5)	204 (12x8.5)	192 (12x8)	192 (12x8)	204 (12x8.5)
	168 (12x7)	168 (12x7)	132 (12x5.5)	156 (12x6.5)	192 (12x8)	168 (12x7)
	204 (12x8.5)	192 (12x8)	147 (10.5x7)	176 (11x8)	168 (12x7)	192 (12x8)
	Total: 768	Total: 708	Total: 675	Total: 692	Total: 651	Total: 768

APPENDIX IV: Quantity of Honey and Pollen collected by the Foraging Honeybees in Control and Treatment

Days after test start (Duration of exposition to the bees)	Quantity of Collected Honey [g]					
	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
4 (4)	50.00	20.00	17.00	36.00	29.00	33.00
8 (4)	63.00	61.00	60.00	51.00	65.00	62.00
11 (4)	61.90	61.10	62.00	64.00	58.70	69.30
12 (1)	47.90	50.30	53.90	58.80	45.90	55.40
15 (3)	61.71	61.67	62.51	69.40	59.12	66.39
18 (3)	63.00	66.80	63.59	64.10	60.00	70.50
20 (2)	51.60	55.00	52.20	50.30	50.10	52.90
22 (2)	19.60	17.98	22.92	21.20	13.07	23.40
26 (4)	37.40	38.50	39.00	32.00	32.70	36.80
29 (3)	29.10	28.00	27.50	23.90	26.00	27.40
32 (3)	46.00	50.20	48.40	36.60	44.60	40.70
34 (2)	37.25	48.10	33.40	34.50	43.20	36.90
36 (2)	52.70	55.40	56.60	37.20	52.40	39.50
40 (4)	47.50	43.00	44.50	12.70	45.60	17.20
45 (6)	33.20	37.10	33.90	29.30	35.80	36.19
Total	701.86	694.15	677.42	621.00	661.19	667.58

Contd' appendix IV: Quantity of Honey and Pollen collected by the Foraging Honeybees in Control and Treatment

Days after test start (Duration of exposition to the bees)	Quantity of Collected Pollen [g]					
	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
8 (8)	0.10	0.08	0.20	0.10	0.10	0.10
8 (2)	2.25	0.73	1.45	2.22	2.19	2.20
12 (4)	0.29	0.16	0.32	0.33	0.04	0.10
15 (7)	2.41	3.68	2.06	6.14	10.06	9.64
18 (6)	0.13	0.05	0.06	0.00	2.00	0.02
22 (7)	1.67	0.50	1.07	2.55	8.26	5.14
22 (4)	0.06	0.08	0.08	0.07	1.75	0.20
29 (7)	0.20	0.70	0.79	0.10	0.88	3.20
29 (7)	0.49	0.78	0.96	0.36	2.35	0.35
34 (5)	0.81	0.89	0.74	0.62	0.82	0.69
36 (7)	2.44	0.80	0.50	7.00	3.90	2.50
36 (2)	0.35	0.32	0.25	0.35	2.27	0.38
45 (9)	0.81	-0.09*	0.98	1.00	2.33	2.43
45 (9)	0.19	0.12	0.16	0.17	2.80	0.11
Total	12.20	8.89	9.62	21.01	39.75	27.06

* balance error

APPENDIX V: Size of Honey Stores over Time in Control and Treatment

The table shows the comb areas of four combs where stored honey was recorded during the evaluations. The first values give the absolute area in cm² which contained honey (taking into account the values of the newly produced comb area from appendix III). The values in parenthesis give the percentage of honey stores at the front and back site of each comb.

The absolute area in cm² containing honey was calculated as follows:

$$\frac{\% \text{ of comb area of both comb sides with cells containing honey} \times \text{comb area in cm}^2 \text{ from appendix III}}{\text{total \% of comb area from both comb sides}}$$

Days after first exposure to treated substrate	Honey deposition area [%combs with honey/total = cm ² combs with honey]					
	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
8	38 (20, 25)	38 (50, 40)	14 (10, 5)	29 (20, 20)	44 (80, 65)	38 (20, 20)
	8 (5, 10)	6 (5, 5)	16 (20, 20)	3 (0, 5)	6 (5, 5)	12 (5, 10)
	0 (0, 0)	2 (5, 0)	0 (0, 0)	0 (0, 0)	17 (15, 20)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 46	Total: 46	Total: 30	Total: 32	Total: 67	Total: 50
14	120 (65, 60)	87 (75, 70)	62 (30, 35)	97 (60, 55)	50 (65, 60)	62 (35, 30)
	34 (20, 20)	42 (25, 25)	63 (40, 35)	35 (25, 20)	42 (30, 20)	29 (15, 15)
	50 (35, 40)	78 (55, 45)	29 (25, 35)	45 (40, 35)	31 (20, 20)	76 (50, 65)
	(0, 0)	3 (10, 0)	0 (0, 0)	0 (0, 0)	40 (50, 40)	0 (0, 0)
	Total: 204	Total: 210	Total: 154	Total: 177	Total: 163	Total: 167
21	168 (90, 85)	115 (80, 80)	67 (40, 30)	134 (85, 75)	79 (85, 75)	106 (60, 50)
	45 (20, 30)	55 (30, 35)	95 (50, 55)	55 (35, 30)	72 (45, 35)	58 (35, 25)
	86 (60, 60)	98 (60, 65)	75 (65, 60)	47 (35, 30)	38 (25, 20)	105 (70, 65)
	0 (0, 0)	13 (5, 35)	0 (0, 0)	0 (0, 0)	69 (65, 50)	2 (10, 0)
	Total: 299	Total: 281	Total: 237	Total: 236	Total: 258	Total: 271
29	163 (85, 85)	115 (80, 80)	72 (40, 35)	143 (85, 85)	32 (65, 0)	120 (65, 60)
	41 (25, 20)	46 (30, 25)	113 (65, 60)	29 (20, 15)	90 (60, 40)	43 (20, 25)
	83 (55, 60)	101 (65, 65)	54 (60, 30)	86 (50, 60)	34 (20, 20)	94 (60, 60)
	0 (0, 0)	2 (5, 0)	0 (0, 0)	0 (0, 0)	69 (60, 55)	0 (0, 0)
	Total: 287	Total: 264	Total: 239	Total: 258	Total: 225	Total: 257
37	173 (90, 90)	122 (85, 85)	106 (50, 60)	139 (80, 85)	47 (85, 10)	139 (70, 75)
	54 (30, 30)	76 (45, 45)	113 (65, 60)	63 (35, 40)	131 (75, 70)	62 (35, 30)
	97 (70, 65)	109 (70, 70)	57 (45, 50)	94 (60, 60)	67 (45, 35)	105 (65, 70)
	1 (5, 0)	2 (5, 0)	0 (0, 0)	0 (0, 0)	78 (65, 65)	0 (0, 0)
	Total: 325	Total: 309	Total: 276	Total: 296	Total: 323	Total: 306
45	144 (80, 70)	125 (80, 80)	58 (25, 35)	122 (75, 70)	69 (90, 50)	125 (70, 60)
	32 (15, 20)	27 (15, 15)	63 (35, 35)	34 (20, 20)	59 (30, 35)	38 (20, 20)
	43 (50, 10)	105 (60, 65)	73 (65, 45)	39 (50, 0)	38 (20, 20)	109 (65, 65)
	10 (10, 5)	17 (10, 10)	4 (5, 5)	5 (5, 5)	80 (55, 40)	4 (5, 0)
	Total: 229	Total: 274	Total: 198	Total: 200	Total: 246	Total: 306
52	110 (65, 50)	101 (50, 70)	38 (25, 15)	63 (40, 35)	47 (85, 10)	71 (35, 35)
	31 (20, 10)	23 (25, 20)	51 (25, 25)	19 (10, 10)	38 (20, 20)	36 (15, 20)
	38 (40, 5)	76 (50, 40)	20 (20, 10)	51 (65, 0)	24 (10, 15)	105 (60, 65)
	15 (5, 10)	34 (15, 20)	7 (5, 5)	0 (0, 0)	63 (40, 35)	14 (10, 5)
	Total: 194	Total: 234	Total: 116	Total: 133	Total: 172	Total: 226

APPENDIX VI: Size of Pollen Stores over Time in Control and Treatment

The table shows the comb areas of four combs where stored pollen was recorded during the evaluations. The first values give the absolute area in cm² which contained pollen (taking into account the values of the newly produced comb area from appendix III). The values in parenthesis give the percentage of pollen stores at the front and back site of each comb.

The absolute area in cm² containing pollen was calculated as follows:

$$\frac{\% \text{ of comb area of both comb sides with cells containing pollen} \times \text{comb area in cm}^2 \text{ from appendix III}}{\text{total \% of comb area from both comb sides}}$$

Days after first exposure to treated substrate	Pollen deposition area [%combs with pollen/total = cm ² combs with pollen]					
	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
8	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	1 (0, 1)
	0 (0, 0)	0 (0, 0)	1 (1, 0)	1 (1, 1)	15 (15, 10)	1 (0, 1)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	2 (5, 0)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 0	Total: 0	Total: 1	Total: 1	Total: 17	Total: 2
14	5 (0, 5)	0 (0, 0)	10 (5, 5)	8 (5, 5)	10 (15, 10)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	4 (5, 0)	4 (0, 5)	4 (0, 5)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	3 (0, 5)	0 (0, 0)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	9 (10, 10)	0 (0, 0)
	Total: 5	Total: 0	Total: 14	Total: 15	Total: 23	Total: 0
21	0 (0, 0)	0 (0, 0)	0 (0, 0)	4 (0, 5)	2 (5, 0)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	9 (5, 5)	5 (0, 5)
	0 (0, 0)	4 (0, 5)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 0	Total: 4	Total: 0	Total: 4	Total: 11	Total: 5
29	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 0	Total: 0	Total: 0	Total: 0	Total: 0	Total: 0
37	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	5 (5, 0)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	13 (10, 5)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 0	Total: 0	Total: 0	Total: 0	Total: 18	Total: 0
45	10 (0, 10)	4 (0, 5)	5 (5, 0)	8 (5, 5)	0 (0, 0)	5 (0, 5)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	9 (10, 0)	0 (0, 0)
	36 (5, 0)	8 (5, 5)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	4 (5, 5)	0 (0, 0)	13 (5, 10)	0 (0, 0)
	Total: 46	Total: 12	Total: 9	Total: 8	Total: 22	Total: 5
52	24 (0, 25)	4 (0, 5)	14 (5, 10)	13 (10, 5)	0 (0, 0)	10 (5, 5)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	17 (15, 5)	9 (10, 0)	0 (0, 0)	4 (5, 0)	0 (0, 0)	17 (20, 0)
	0 (0, 0)	0 (0, 0)	4 (5, 0)	0 (0, 0)	25 (10, 20)	0 (0, 0)
	Total: 41	Total: 13	Total: 18	Total: 17	Total: 25	Total: 27

APPENDIX VII: Weight increase of bee hives in Control and Treatment

Days after test start (Duration of exposition to the bees)	Total Hive Weight [g]					
	Control	Control	Control	Treatment	Treatment	Treatment
	1a	1b	1c	3a	3b	3c
0	940	960	950	955	910	925
8	905*	920*	930*	920*	880*	890*
14	1010*	1010*	1010*	1035*	965*	985*
21	1075*	1080*	1075*	1085*	1010*	1060*
29	1070*	1090*	1065*	1050*	1000*	1035*
36	1100*	1135*	1100*	1060*	1030*	1045*
43	1180	1225	1160	1120	1125	1125
Total weight gain [g]	240	265	210	165	215	200
Weight gain [%]**	25.53	27.60	22.11	17.28	23.63	21.62

* weight including weight of the hive feeder

** In relation to the initial weight.

APPENDIX VIII: Queen Egg Laying Activity in Control and Treatment

The table shows the comb areas of four combs where an egg was recorded during the evaluations. The first values give the absolute area in cm² which contained an egg (taking into account the values of the newly produced comb area from appendix III). The values in parenthesis give the percentage of eggs at the front and back site of each comb.

The absolute area in cm² containing cells with eggs was calculated as follows:

$$\frac{\% \text{ of comb area of both comb sides with cells containing eggs} \times \text{comb area in cm}^2 \text{ from appendix III}}{\text{total \% of comb area from both comb sides}}$$

Days after first exposure to treated substrate	Egg deposition activity [% combs with eggs/total=cm ² combs with eggs]					
	Control	Control	Control	Treatment	Treatment	Treatment
	1a	1b	1c	3a	3b	3c
8	92 (60, 50)	29 (30, 40)	90 (50, 50)	65 (50, 40)	0 (0, 0)	77 (40, 40)
	73 (70, 65)	88 (70, 80)	59 (40, 50)	54 (40, 50)	60 (50, 50)	109 (70, 70)
	0 (0, 0)	48 (60, 50)	0 (0, 0)	0 (0, 0)	48 (60, 40)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	16 (50, 30)	0 (0, 0)
	Total: 165	Total: 165	Total: 149	Total: 119	Total: 124	Total: 186
14	5 (0, 5)	0 (0, 0)	58 (30, 30)	29 (20, 15)	0 (0, 0)	53 (35, 20)
	67 (40, 40)	84 (50, 50)	59 (35, 35)	59 (40, 35)	92 (50, 60)	96 (50, 50)
	43 (35, 30)	59 (40, 35)	0 (0, 0)	3 (0, 5)	78 (50, 50)	3 (0, 5)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 115	Total: 143	Total: 117	Total: 91	Total: 170	Total: 149
21	0 (0, 0)	0 (0, 0)	48 (25, 25)	0 (0, 0)	0 (0, 0)	10 (5, 5)
	50 (30, 25)	63 (35, 40)	45 (20, 30)	29 (15, 20)	50 (25, 30)	53 (30, 25)
	22 (20, 10)	27 (20, 15)	0 (0, 0)	40 (30, 25)	38 (20, 25)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 72	Total: 90	Total: 93	Total: 69	Total: 88	Total: 63
29	0 (0, 0)	0 (0, 0)	29 (25, 5)	0 (0, 0)	0 (0, 0)	24 (15, 10)
	45 (30, 20)	55 (30, 35)	18 (10, 10)	34 (20, 20)	27 (10, 20)	48 (20, 30)
	0 (0, 0)	4 (5, 0)	0 (0, 0)	16 (10, 10)	34 (10, 30)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 45	Total: 59	Total: 47	Total: 50	Total: 61	Total: 72
37	0 (0, 0)	0 (0, 0)	38 (20, 20)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	68 (30, 45)	38 (25, 20)	18 (10, 10)	38 (10, 35)	9 (5, 5)	53 (20, 35)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	42 (25, 25)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 68	Total: 38	Total: 56	Total: 38	Total: 51	Total: 53
45	0 (0, 0)	0 (0, 0)	48 (25, 25)	4 (0, 5)	0 (0, 0)	38 (10, 30)
	63 (35, 35)	72 (40, 40)	68 (40, 35)	38 (25, 20)	81 (50, 40)	77 (40, 40)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	58 (30, 30)	21 (15, 10)
	102 (75, 80)	122 (70, 75)	24 (35, 25)	54 (50, 50)	59 (35, 35)	125 (80, 75)
	Total: 165	Total: 194	Total: 140	Total: 96	Total: 198	Total: 261
52	34 (15, 20)	0 (0, 0)	38 (20, 20)	55 (30, 35)	0 (0, 0)	77 (40, 35)
	46 (20, 25)	50 (30, 25)	36 (15, 20)	48 (30, 20)	67 (30, 40)	46 (20, 25)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	58 (30, 30)	0 (0, 0)
	97 (45, 50)	48 (20, 30)	56 (40, 35)	79 (50, 40)	0 (0, 0)	58 (30, 30)
	Total: 177	Total: 98	Total: 130	Total: 182	Total: 125	Total: 181

APPENDIX IX: Abundance of Honeybee Larvae (non-capped brood) in Control and Treatment

The table shows the comb areas of four combs where larvae were recorded during the evaluations. The first values give the absolute area in cm² which contained larvae (taking into account the values of the newly produced comb area from appendix III). The values in parenthesis give the percentage of larvae at the front and back site of each comb.

The absolute area in cm² containing cells with larvae was calculated as follows:

$$\frac{\% \text{ of comb area of both comb sides with cells containing larvae} \times \text{comb area in cm}^2 \text{ from appendix III}}{\text{total \% of comb area from both comb sides}}$$

Days after first exposure to treated substrate	Larval abundance [% combs with larvae/cm ² combs with larvae]					
	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
8	0 (0, 0)	0 (0, 0)	27 (20, 10)	0 (0, 0)	0 (0, 0)	34 (25, 10)
	0 (0, 0)	6 (5, 5)	0 (0, 0)	3 (0, 5)	0 (0, 0)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 0	Total: 8	Total: 27	Total: 3	Total: 0	Total: 34
14	0 (0, 0)	0 (0, 0)	14 (10, 5)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	34 (25, 15)	8 (5, 5)	8 (5, 5)	12 (10, 5)	21 (15, 10)	24 (15, 10)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	31 (15, 25)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 34	Total: 8	Total: 22	Total: 12	Total: 52	Total: 24
21	0 (0, 0)	0 (0, 0)	10 (5, 5)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	14 (5, 10)	13 (10, 5)	14 (10, 5)	29 (20, 15)	27 (15, 15)	29 (15, 15)
	0 (0, 0)	4 (5, 0)	0 (0, 0)	0 (0, 0)	29 (15, 20)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 14	Total: 17	Total: 24	Total: 29	Total: 56	Total: 29
29	0 (0, 0)	0 (0, 0)	14 (5, 10)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	9 (5, 5)	8 (5, 5)	0 (0, 0)	8 (5, 5)	9 (5, 5)	14 (5, 10)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	8 (5, 5)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 9	Total: 8	Total: 14	Total: 8	Total: 17	Total: 14
32	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	9 (5, 5)	8 (5, 5)	9 (5, 5)	8 (5, 5)	5 (0, 5)	10 (5, 5)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	8 (5, 5)	0 (0, 0)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	Total: 9	Total: 8	Total: 9	Total: 8	Total: 13	Total: 10
45	0 (0, 0)	0 (0, 0)	48 (25, 25)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	59 (30, 35)	59 (35, 30)	14 (5, 10)	34 (15, 25)	9 (5, 5)	48 (25, 25)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	53 (20, 35)	0 (0, 0)
	0 (0, 0)	13 (10, 5)	0 (0, 0)	0 (0, 0)	0 (0, 0)	16 (5, 15)
	Total: 59	Total: 72	Total: 62	Total: 34	Total: 62	Total: 64
52	0 (0, 0)	0 (0, 0)	24 (10, 15)	0 (0, 0)	0 (0, 0)	36 (10, 25)
	46 (25, 20)	27 (15, 15)	36 (15, 20)	24 (15, 10)	43 (25, 20)	36 (25, 10)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	24 (15, 10)	0 (0, 0)
	46 (25, 20)	43 (20, 25)	19 (10, 15)	75 (35, 50)	0 (0, 0)	48 (25, 25)
	Total: 92	Total: 70	Total: 79	Total: 99	Total: 67	Total: 120

Honeybee Semifield Test Imidacloprid

APPENDIX X: Abundance of Honeybee Pupae (capped brood) in Control and Treatment

The table shows the comb areas of four combs where pupae were recorded during the evaluations. The first values give the absolute area in cm² which contained pupae (taking into account the values of the newly produced comb area from appendix III). The values in parenthesis give the percentage of pupae at the front and back site of each comb.

The absolute area in cm² containing cells with pupae was calculated as follows:

$$\frac{\% \text{ of comb area of both comb sides with cells containing pupae} \times \text{comb area in cm}^2 \text{ from appendix III}}{\text{total \% of comb area from both comb sides}}$$

Days after first exposure to treated substrate	Pupal abundance [% combs with pupae/ total cm ² combs with pupae]					
	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
8	0 (0, 0) 0 (0, 0) 0 (0, 0) 0 (0, 0) Total: 0	0 (0, 0) 0 (0, 0) 0 (0, 0) 0 (0, 0) Total: 0	0 (0, 0) 0 (0, 0) 0 (0, 0) 0 (0, 0) Total: 0	0 (0, 0) 0 (0, 0) 0 (0, 0) 0 (0, 0) Total: 0	0 (0, 0) 0 (0, 0) 0 (0, 0) 0 (0, 0) Total: 0	0 (0, 0) 0 (0, 0) 0 (0, 0) 0 (0, 0) Total: 0
14	0 (0, 0) 8 (5, 5) 0 (0, 0) 0 (0, 0) Total: 8	0 (0, 0) 13 (5, 10) 0 (0, 0) 0 (0, 0) Total: 13	19 (10, 10) 0 (0, 0) 0 (0, 0) 0 (0, 0) Total: 19	0 (0, 0) 16 (10, 10) 0 (0, 0) 0 (0, 0) Total: 16	0 (0, 0) 0 (0, 0) 0 (0, 0) 0 (0, 0) Total: 0	19 (10, 10) 10 (5, 5) 0 (0, 0) 0 (0, 0) Total: 29
21	0 (0, 0) 36 (20, 20) 0 (0, 0) 0 (0, 0) Total: 36	0 (0, 0) 29 (20, 15) 0 (0, 0) 0 (0, 0) Total: 29	38 (20, 20) 0 (0, 0) 0 (0, 0) 0 (0, 0) Total: 38	0 (0, 0) 38 (25, 20) 0 (0, 0) 0 (0, 0) Total: 38	0 (0, 0) 9 (5, 5) 42 (25, 25) 0 (0, 0) Total: 51	38 (20, 20) 38 (20, 20) 0 (0, 0) 0 (0, 0) Total: 76
29	0 (0, 0) 32 (20, 15) 0 (0, 0) 0 (0, 0) Total: 32	0 (0, 0) 17 (10, 10) 0 (0, 0) 0 (0, 0) Total: 17	29 (15, 15) 0 (0, 0) 0 (0, 0) 0 (0, 0) Total: 29	0 (0, 0) 25 (10, 20) 0 (0, 0) 0 (0, 0) Total: 25	0 (0, 0) 18 (10, 10) 50 (35, 25) 0 (0, 0) Total: 68	0 (0, 0) 38 (20, 20) 0 (0, 0) 0 (0, 0) Total: 38
37	0 (0, 0) 9 (5, 5) 0 (0, 0) 0 (0, 0) Total: 9	0 (0, 0) 8 (5, 5) 0 (0, 0) 0 (0, 0) Total: 8	0 (0, 0) 9 (5, 5) 0 (0, 0) 0 (0, 0) Total: 9	0 (0, 0) 8 (5, 5) 0 (0, 0) 0 (0, 0) Total: 8	0 (0, 0) 0 (0, 0) 9 (5, 5) 0 (0, 0) Total: 9	0 (0, 0) 10 (5, 5) 0 (0, 0) 0 (0, 0) Total: 10
45	0 (0, 0) 9 (5, 5) 0 (0, 0) 0 (0, 0) Total: 9	0 (0, 0) 5 (0, 5) 0 (0, 0) 0 (0, 0) Total: 5	14 (10, 5) 0 (0, 0) 0 (0, 0) 0 (0, 0) Total: 14	0 (0, 0) 8 (5, 5) 0 (0, 0) 0 (0, 0) Total: 8	0 (0, 0) 9 (5, 5) 34 (20, 15) 0 (0, 0) Total: 43	0 (0, 0) 14 (5, 10) 0 (0, 0) 0 (0, 0) Total: 14
52	0 (0, 0) 77 (35, 40) 0 (0, 0) 36 (20, 15) Total: 113	0 (0, 0) 54 (25, 35) 0 (0, 0) 38 (20, 20) Total: 92	72 (40, 35) 77 (40, 35) 0 (0, 0) 0 (0, 0) Total: 149	0 (0, 0) 86 (40, 50) 0 (0, 0) 0 (0, 0) Total: 86	0 (0, 0) 38 (20, 20) 72 (35, 40) 0 (0, 0) Total: 110	0 (0, 0) 82 (40, 40) 0 (0, 0) 43 (25, 20) Total: 125

APPENDIX XI: Population Development in Control and Treatment

The table shows the comb area of four combs which was occupied by adult honeybees during the evaluations. The first values give the absolute area in cm² which was covered by adult bees (taking into account the values of the newly produced comb area from appendix III). The values in parenthesis give the percentage of area covered by adult bees at the front and back site of each comb.

The absolute area in cm² covered by adult bees was calculated as follows:

$$\frac{\% \text{ of comb area of both comb sides covered by adult bees} \times \text{comb area in cm}^2 \text{ from appendix III}}{\text{total \% of comb area from both comb sides}}$$

Days after first exposure to treated substrate	Population density [% occupied combs/total=cm ² occupied comb area]					
	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
8	92 (50, 60)	55 (50, 80)	117 (60, 70)	86 (50, 70)	24 (50, 30)	86 (50, 40)
	59 (50, 60)	53 (50, 40)	106 (80, 80)	79 (70, 60)	60 (60, 40)	86 (70, 40)
	0 (0, 0)	31 (40, 30)	0 (0, 0)	10 (50, 50)	43 (60, 30)	18 (30, 30)
	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	14 (30, 40)	0 (0, 0)
	Total: 151	Total: 139	Total: 223	Total: 175	Total: 141	Total: 190
14	96 (40, 60)	60 (40, 60)	86 (60, 30)	50 (30, 30)	48 (40, 80)	96 (35, 65)
	76 (40, 50)	89 (35, 70)	84 (70, 30)	86 (50, 60)	76 (50, 40)	110 (80, 35)
	40 (40, 20)	70 (40, 50)	26 (30, 25)	42 (40, 30)	51 (35, 30)	86 (30, 60)
	0 (0, 0)	5 (10, 5)	0 (0, 0)	0 (0, 0)	24 (15, 40)	0 (5, 15)
	Total: 212	Total: 224	Total: 196	Total: 178	Total: 199	Total: 292
21	53 (30, 25)	29 (20, 20)	134 (60, 80)	42 (30, 20)	15 (20, 10)	77 (35, 45)
	108 (40, 80)	105 (65, 60)	90 (35, 65)	113 (70, 65)	90 (40, 60)	96 (40, 60)
	79 (60, 50)	86 (50, 60)	42 (40, 30)	61 (50, 35)	84 (50, 50)	82 (65, 40)
	19 (30, 50)	13 (10, 30)	0 (0, 0)	8 (5, 30)	42 (40, 30)	6 (20, 5)
	Total: 259	Total: 233	Total: 266	Total: 224	Total: 231	Total: 261
29	48 (30, 20)	61 (25, 60)	125 (80, 50)	59 (40, 30)	5 (0, 10)	58 (20, 40)
	81 (40, 50)	84 (50, 50)	90 (40, 60)	84 (50, 50)	63 (20, 50)	86 (60, 30)
	72 (60, 40)	98 (60, 65)	18 (20, 10)	55 (30, 40)	71 (25, 60)	86 (70, 40)
	4 (5, 10)	13 (25, 5)	0 (0, 0)	4 (15, 0)	39 (30, 35)	1 (0, 5)
	Total: 205	Total: 256	Total: 233	Total: 202	Total: 178	Total: 231
37	48 (20, 30)	29 (20, 20)	58 (30, 30)	42 (30, 20)	10 (10, 10)	38 (20, 20)
	59 (35, 30)	55 (40, 25)	63 (40, 30)	76 (40, 50)	54 (20, 40)	58 (30, 30)
	43 (35, 25)	47 (35, 25)	42 (30, 40)	39 (20, 30)	63 (30, 45)	43 (25, 30)
	1 (0, 5)	9 (20, 0)	0 (0, 0)	0 (0, 0)	21 (10, 25)	0 (0, 0)
	Total: 151	Total: 140	Total: 163	Total: 157	Total: 148	Total: 139
45	62 (50, 15)	70 (30, 60)	91 (50, 45)	63 (25, 50)	10 (10, 10)	82 (35, 50)
	99 (50, 50)	90 (50, 50)	72 (40, 40)	101 (60, 60)	81 (50, 40)	120 (60, 65)
	65 (55, 35)	76 (40, 50)	43 (40, 25)	31 (30, 10)	106 (45, 65)	92 (50, 60)
	89 (65, 70)	59 (35, 35)	36 (40, 50)	43 (30, 50)	67 (35, 45)	81 (50, 50)
	Total: 315	Total: 295	Total: 242	Total: 238	Total: 264	Total: 375
52	58 (10, 50)	29 (15, 20)	34 (15, 20)	76 (40, 50)	17 (25, 10)	87 (25, 60)
	92 (20, 70)	81 (30, 60)	92 (30, 60)	96 (40, 60)	77 (50, 30)	82 (30, 50)
	34 (15, 25)	25 (20, 10)	20 (20, 10)	27 (35, 0)	86 (60, 30)	29 (15, 20)
	82 (20, 60)	48 (20, 30)	37 (20, 30)	66 (35, 40)	42 (15, 35)	62 (25, 40)
	Total: 266	Total: 183	Total: 183	Total: 265	Total: 222	Total: 260

APPENDIX XII: Activity Pattern of Foraging Honeybees in Control and Treatment

The table gives the average number of honeybees which were recorded during the daily 5 minutes observation periods at the tunnel roof

Days after first exposure to treated substrate	Number of honeybees found at the tunnel roof					
	Control	Control	Control	Treatment	Treatment	Treatment
	1a	1b	1c	3a	3b	3c
4	2	2	1	7	4	3
5	0	1	1	0	1	1
6	1	0	0	0	0	0
7	0	1	0	0	0	0
8	3	4	1	5	7	2
11	3	4	1	2	3	1
12	4	3	5	5	9	6
13	5	3	1	5	5	4
14	6	5	5	3	4	5
15	5	4	2	3	5	6
18	5	4	4	4	6	5
19	5	5	5	4	5	6
20	0	0	0	1	0	0
21	0	1	0	1	0	1
22	4	3	2	3	0	4
25	2	2	1	4	7	2
26	4	5	4	5	3	5
27	7	1	1	0	0	0
28	3	3	3	2	2	3
32	3	5	4	5	5	3
33	2	6	1	4	6	2
34	1	1	2	3	3	2
35	3	3	1	5	3	3
36	4	3	2	3	3	1
39	3	5	4	2	3	5
40	3	3	5	3	5	2
41	4	4	3	4	4	5
42	3	5	4	5	4	4
Total	79	98	63	82	91	81

Honeybee Semifield Test Imidacloprid

Cont' Appendix XII: Activity Pattern of Foraging Honeybees in Control and Treatment

The table gives the average number of honeybees which were recorded during the daily 5 minutes observation periods at the pollen feeder.

Days after first exposure to treated substrate	Number of honeybees found at the pollen feeder					
	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
11	0	0	0	0	0	0
12	0	0	0	0	0	0
13	0	0	0	0	0	0
14	0	0	0	0	0	0
15	0	0	0	0	0	0
18	0	0	0	0	0	0
19	0	0	0	1	1	0
20	0	1	0	0	0	0
21	1	0	0	0	1	0
22	1	0	1	1	2	1
25	1	0	0	0	1	0
26	2	1	1	1	3	1
27	1	1	1	1	1	1
28	1	0	0	0	3	1
32	1	2	2	2	2	1
33	0	1	1	1	1	1
34	1	1	1	1	1	0
35	2	2	2	2	2	1
36	2	2	3	3	3	2
39	3	3	3	2	2	2
40	2	4	2	2	3	3
41	1	1	1	1	2	1
42	1	1	2	1	2	2
Total	20	20	20	22	30	17

Dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt op grond van een wettelijke verplichting tot openbaarmaking. Op dit document kunnen rechten van derden rusten, waaronder intellectuele eigendomsrechten en/of auteursrechten. Publicatie, verspreiding, vermenigvuldiging, commerciële exploitatie en gebruik van dit document of de inhoud hiervan zonder de toestemming van de rechthebbende van dit document kan derhalve verboden zijn en een inbreuk opleveren van de rechten van de rechthebbende.

This document is not the property of the Ctgb and only provided based on mandatory freedom of information requirements. The document may be subject to rights such as intellectual property and copy rights of third parties. Consequently, any publication, distribution, reproduction and/or publishing of this document may therefore be prohibited and violate the rights of its owner.

Cont' Appendix XII: Activity Pattern of Foraging Honeybees in Control and Treatment

The table gives the average number of honeybees which were recorded during the daily 5 minutes observation periods at the honey feeder.

Days after first exposure to treated substrate	Number of honeybees found at the honey feeder					
	Control 1a	Control 1b	Control 1c	Treatment 3a	Treatment 3b	Treatment 3c
4	2	6	2	1	1	6
5	6	7	4	5	4	6
6	6	8	4	8	9	8
7	5	7	6	1	8	8
8	4	4	5	7	2	1
11	6	6	5	5	9	8
12	9	12	9	10	11	10
13	10	10	10	8	12	10
14	12	11	12	10	12	12
15	11	12	12	13	11	13
18	8	9	11	8	10	12
19	9	10	10	9	10	10
20	2	2	3	5	1	1
21	8	8	7	8	9	8
22	7	8	8	8	8	8
25	5	4	6	6	5	4
26	7	8	9	9	7	7
27	5	5	6	6	6	5
28	5	6	6	5	6	6
32	8	9	8	8	10	9
33	9	10	9	6	9	9
34	10	11	12	12	13	8
35	10	10	10	10	11	10
36	9	11	8	10	10	10
39	10	11	10	12	11	11
40	9	10	9	8	10	11
41	5	5	7	4	7	5
42	7	7	8	8	8	4
Total	204	227	216	204	230	220

APPENDIX XIII: Analytical Report - Evaluation of the Effects of Residues of Imidacloprid FS 600 in Maize Pollen from Dressed Seeds on Honeybees (*Apis Mellifera*) in the Semifield

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

2001-11-28
Report No.: MR-547/01
Page 1 of 11

STUDY TITLE

Evaluation of the Effects of Residues of Imidacloprid FS 600 in Maize Pollen from Dressed Seeds on Honeybees (*Apis Mellifera*) in the Semifield

Author

[REDACTED]

Testing Facility

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

Analytical Completion Date

2001-11-28

Study Number

E 319 2046-5

STUDY TITLE

Evaluation of the Effects of Residues of Imidacloprid FS 600 in Maize Pollen from Dressed Seeds on Honeybees (*Apis Mellifera*) in the Semifield

Author

[REDACTED]

Testing Facility

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

Analytical Completion Date

2001-11-28

Study Number

E 319 2046-5

CERTIFICATION OF AUTHENTICITY



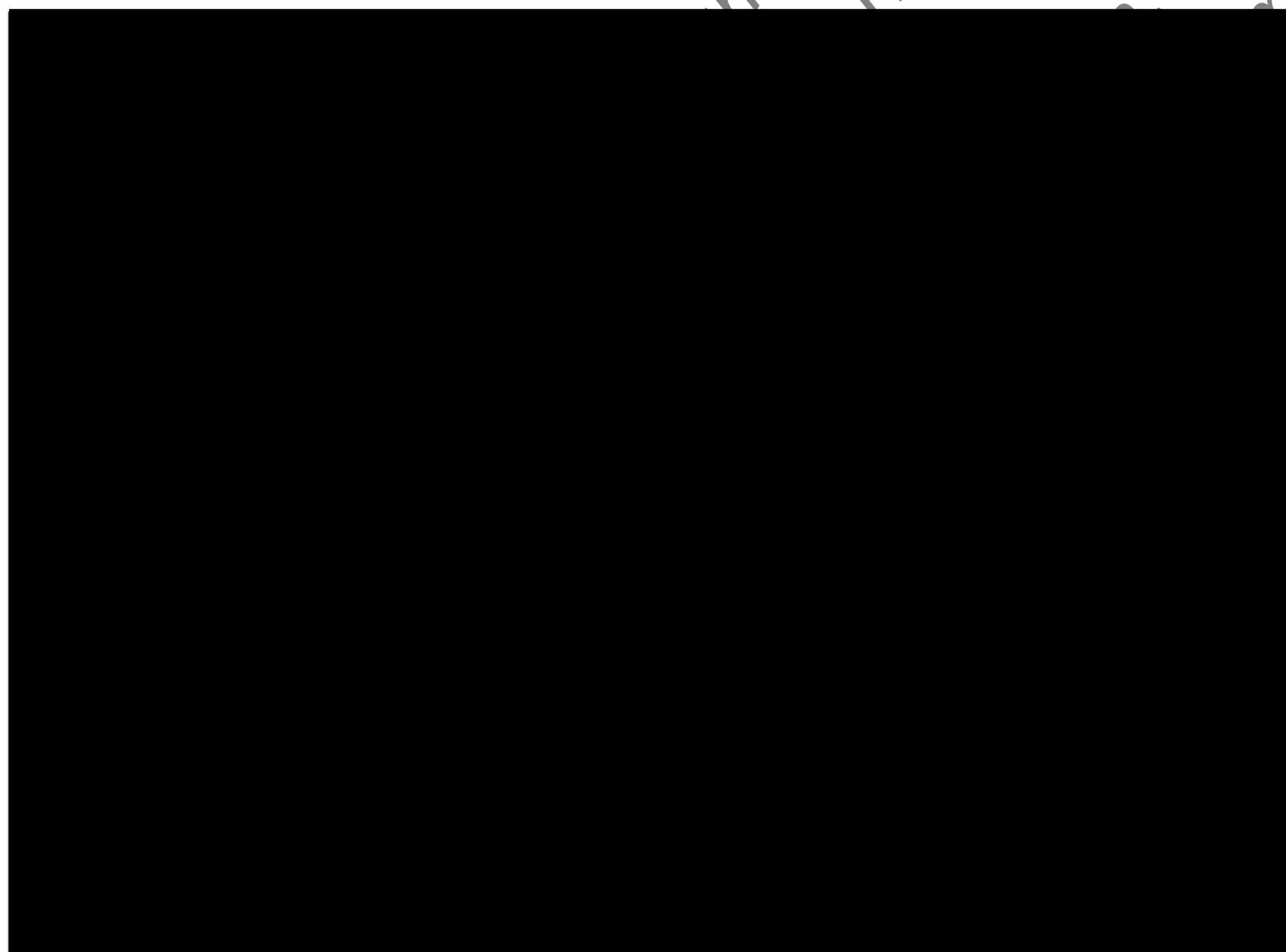
Author and
Responsible Analyst

2001-11-28
Date: yyyy-mm-dd

Head of Test Facility

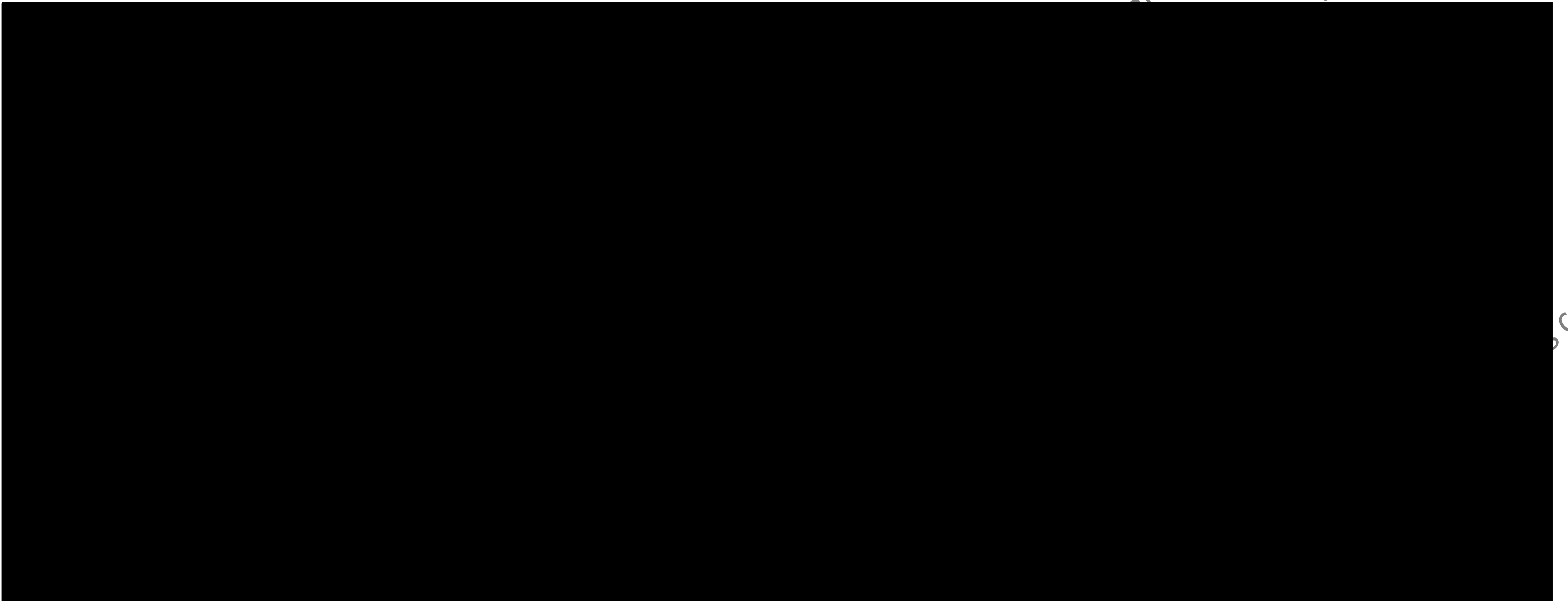
2001-11-30
Date: yyyy-mm-dd

Inquiries should be directed to:

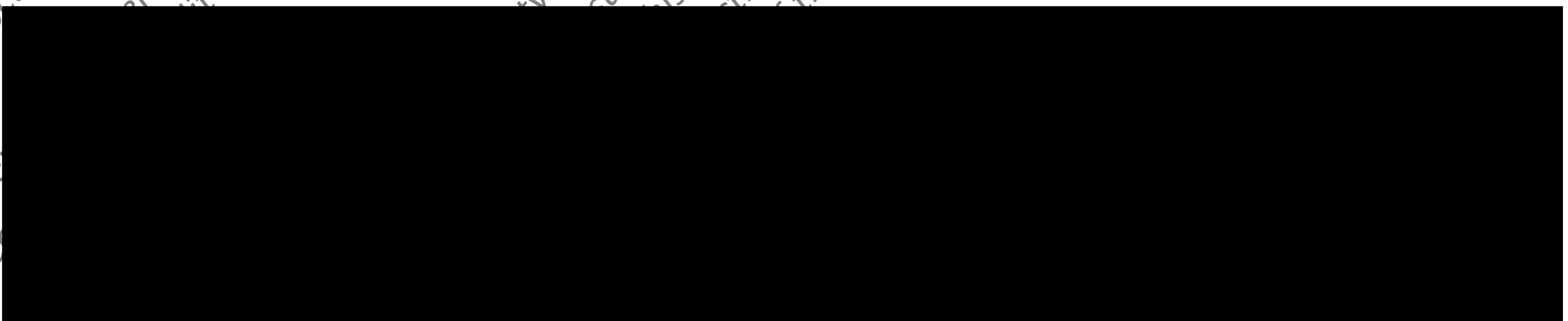


Dit document is geen eigendom van Bayer AG en wordt uitsluitend ter beschikking gesteld op grond van een wettelijke verplichting tot openbaarmaking. Het document is niet de eigendom van Bayer AG en wordt uitsluitend ter beschikking gesteld op grond van een wettelijke verplichting tot openbaarmaking. The document is not the property of the Ctgb and only provided based on mandatory freedom of information requirements. Furthermore, this document may fall under a regulatory data protection and copy rights of third parties. Voorts kan dit document onderworpen zijn aan een inbreuk opleveren van de rechten van deze rechthebbende. Publicatie, verspreiding, vermenigvuldiging, comitering, distributie en gebruik van dit document of de inhoud hiervan zond u tegen de toestemming van de rechthebbende. Furthermore, reproduction and/or publishing and any commercial exploitation and use of this document or its contents is prohibited and violate the rights of its owner.

STATEMENT OF COMPLIANCE



Signature:



Dit document is geen eigendom van het Ctgb en wordt beschikbaar gemaakt op grond van de wet van 2010-2011, artikel 17, lid 2, onder a. Het is niet toegestaan dit document te kopiëren, te verspreiden of openbaar te maken. Voorts kan dit document onder een regeling omtrent gegevensbescherming vallen. Het gebruik van dit document kan derhalve verboden zijn en een inbreuk opleveren op de rechten van de afzender.

This document is not the property of the Ctgb and is only provided based on mandatory freedom of information legislation. It is not permitted to copy, disseminate or make this document public. Furthermore, this document may fall under a regulatory data protection regime and its use may therefore be prohibited and violate the rights of the sender.

Publicatie, verspreiding of openbaar maken van dit document is niet toegestaan. Voorts kan dit document onder een regeling omtrent gegevensbescherming vallen. Het gebruik van dit document kan derhalve verboden zijn en een inbreuk opleveren op de rechten van de afzender.

Consequently, any publication, dissemination or public use of this document is not permitted. Furthermore, this document may fall under a regulatory data protection regime and its use may therefore be prohibited and violate the rights of the sender.

TABLE OF CONTENT

	Page No.
Study Title	1
Certification of Authenticity	2
Statement of Compliance	3
Table of Content	4
1 Introduction	5
2 Schedule	5
3 Sample List	5
3.1 <i>Untreated and Treated Plot Samples</i>	5
4 Results	6
4.1 <i>Results from the Pollen Samples from the Untreated and Treated Study Plots</i>	6
4.2 <i>Results from the Honey Samples that was Provided as Carbohydrate Source for the Honeybees</i>	6
5. Experimental	7
5.1 <i>Reference Substances</i>	7
5.2 <i>Residue Analytical Methodology</i>	8
5.2.1 <i>Extraction and Sample Clean-up</i>	8
5.2.2 <i>ChemElut® Column Clean-up</i>	8
5.2.3 <i>Silica Gel Column Clean-up</i>	9
5.3 <i>HPLC-MS/MS Determination of Imidacloprid and Metabolites</i>	10
5.3.1 <i>Measuring Equipment and HPLC Conditions:</i>	10
5.3.2 <i>MS/MS-Detection</i>	11

Dit document is geen eigendom van het CtG en wordt beschouwd als openbaar gemaakt op grond van een wettelijke verplichting tot openbaarmaking. Op dit document kunnen rechten van derden rusten. Voor het gebruik van dit document onder een andere wettelijke bescherming vallen. Het document is niet de eigendom van derden en kan anderszins inbreuk opleveren van de rechten van deze rechthebbende.

Publicatie, verspreiding, vermenigvuldiging, verspreiding, verspreiding of anderszins openbaar maken van dit document kan anderszins inbreuk opleveren van de rechten van deze rechthebbende.

This document is not the property of the CtG and is only provided based on mandatory freedom of information requirements. Consequently, any publication, distribution, reproduction and use of this document or its contents without the permission of the owner of this document may therefore be prohibited and violate the rights of its owner.

1 INTRODUCTION

Maize pollen samples obtained from trial stations of South Africa and Brazil were analysed for residues of Imidacloprid and its Olefin- and Hydroxy metabolites. Additionally honey that was provided as carbohydrate source for the honeybees was analysed for contamination's of Imidacloprid and its metabolites. The results are summarised in the tables 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 SCHEDULE

The experimental work was performed during the following time period:

Signature of study protocol: 2001-02-16
Start of analytical phase: 2001-03-14
End of analytical phase: 2001-06-05

3 SAMPLE LIST

3.1 Untreated and Treated Plot Samples

The following samples were collected from the field trials:

Sample Material	Origin of Sample	Sample Typ	Treatment	Sample Weight [g]
Maize Pollen No. 1	South Africa	Control	-	196.52
Maize Pollen No. 2	South Africa	Treated	NTN 33893 FS 600	128.90
Maize Pollen No. 1	Brazil	Control	-	465.58
Maize Pollen No. 2	Brazil	Control	-	426.90
Maize Pollen No. 3	Brazil	Treated	NTN 33893 FS 600	509.54
Sunflower Honey 1	Honey Müngersdorf in Cologne	Control	-	20
Sunflower Honey 2	Honey Müngersdorf in Cologne	Control	-	20

4 RESULTS

4.1 Results from the Pollen Samples from the Untreated and Treated Study Plots

	Sample Material	Treatment	Hydroxy- Imidacloprid [mg/kg] *	Olefin- Imidacloprid [mg/kg] *	Imidacloprid [mg/kg] *
South Africa	Maize Pollen No. K-01	Control	n.d./n.d.	n.d./n.d.	0.032/0.032
	Maize Pollen No. K-02	Control	n.d./n.d.	n.d./n.d.	0.032/0.033
	Maize Pollen No. B-01	NTN 33893 FS 600	n.d./n.d.	n.d./n.d.	0.046/0.044
	Maize Pollen No. B-02	NTN 33893 FS 600	n.d./n.d.	n.d./n.d.	0.032/0.032
Brazil	Maize Pollen No. K-1A	Control	n.d./n.d.	n.d./n.d.	n.d./n.d.
	Maize Pollen No. K-1B	Control	n.d./n.d.	n.d./n.d.	n.d./n.d.
	Maize Pollen No. K-2A	Control	n.d./n.d.	n.d./n.d.	n.d./n.d.
	Maize Pollen No. K-2B	Control	n.d./n.d.	n.d./n.d.	n.d./n.d.
	Maize Pollen No. B-01	NTN 33893 FS 600	n.d./n.d.	n.d./n.d.	n.d./n.d.
	Maize Pollen No. B-02	NTN 33893 FS 600	n.d./n.d.	n.d./n.d.	n.d./n.d.

*: 1. Measurement/2. Measurement

4.2 Results from the Honey Samples that was Provided as Carbohydrate Source for the Honeybees

	Sample Material	Treatment	Hydroxy- Imidacloprid [mg/kg]*	Olefin- Imidacloprid [mg/kg]*	Imidacloprid [mg/kg]
Sunflower	Honey 1 A	Control	n.d./n.d.	n.d./n.d.	n.d./n.d.
Sunflower	Honey 1 B	Control	n.d./n.d.	n.d./n.d.	n.d./n.d.
Sunflower	Honey 2 A	Control	n.d./n.d.	n.d./n.d.	n.d./n.d.
Sunflower	Honey 2 B	Control	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,

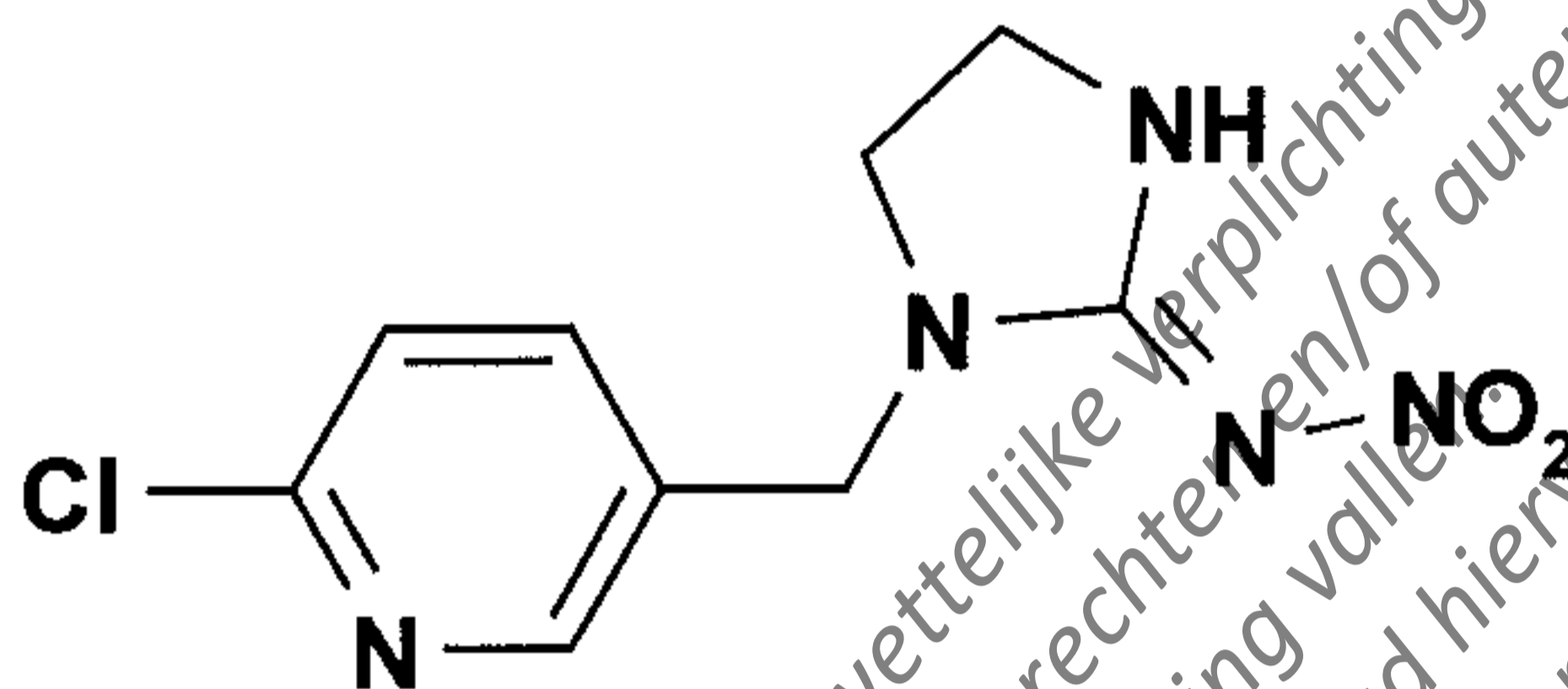
n.d.: Residues below the limit of detection

5. EXPERIMENTAL

5.1 Reference Substances

Imidacloprid

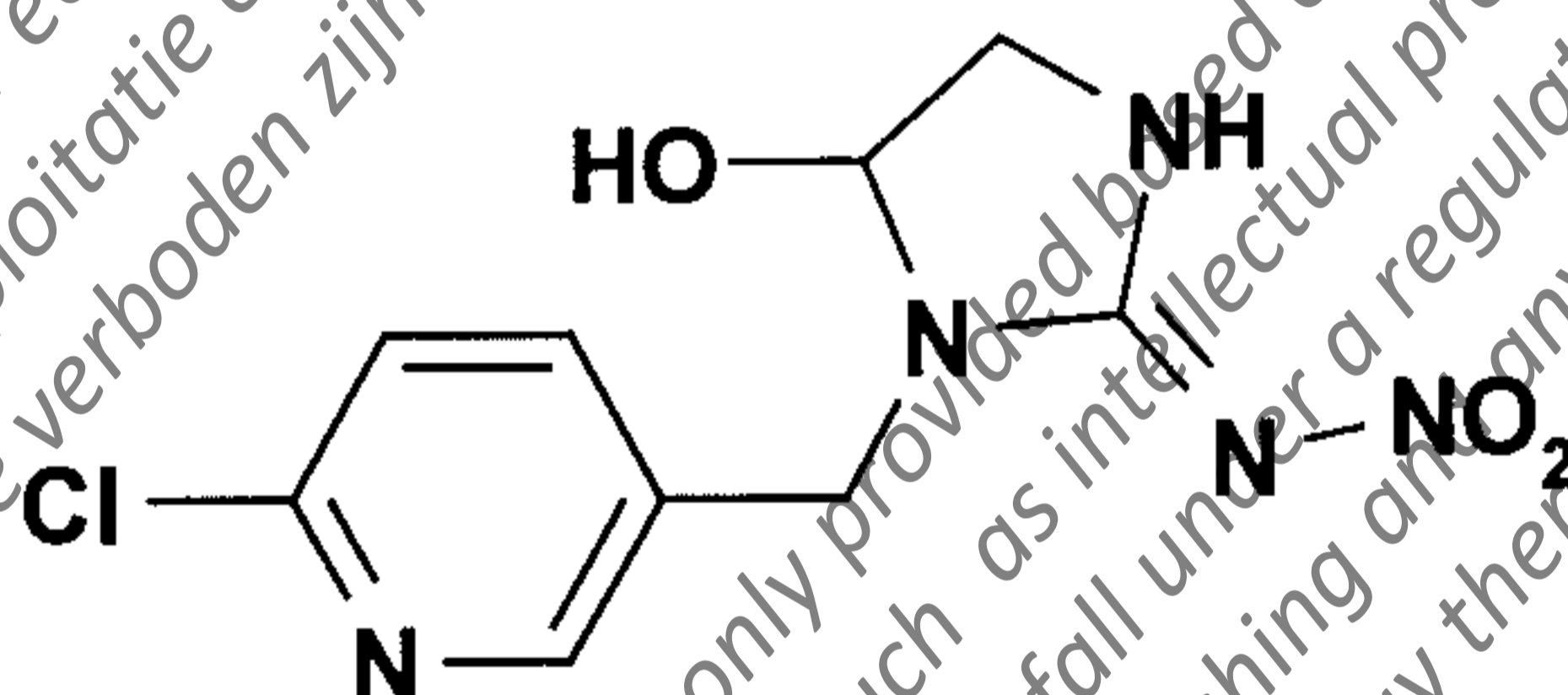
Structural formula:



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)

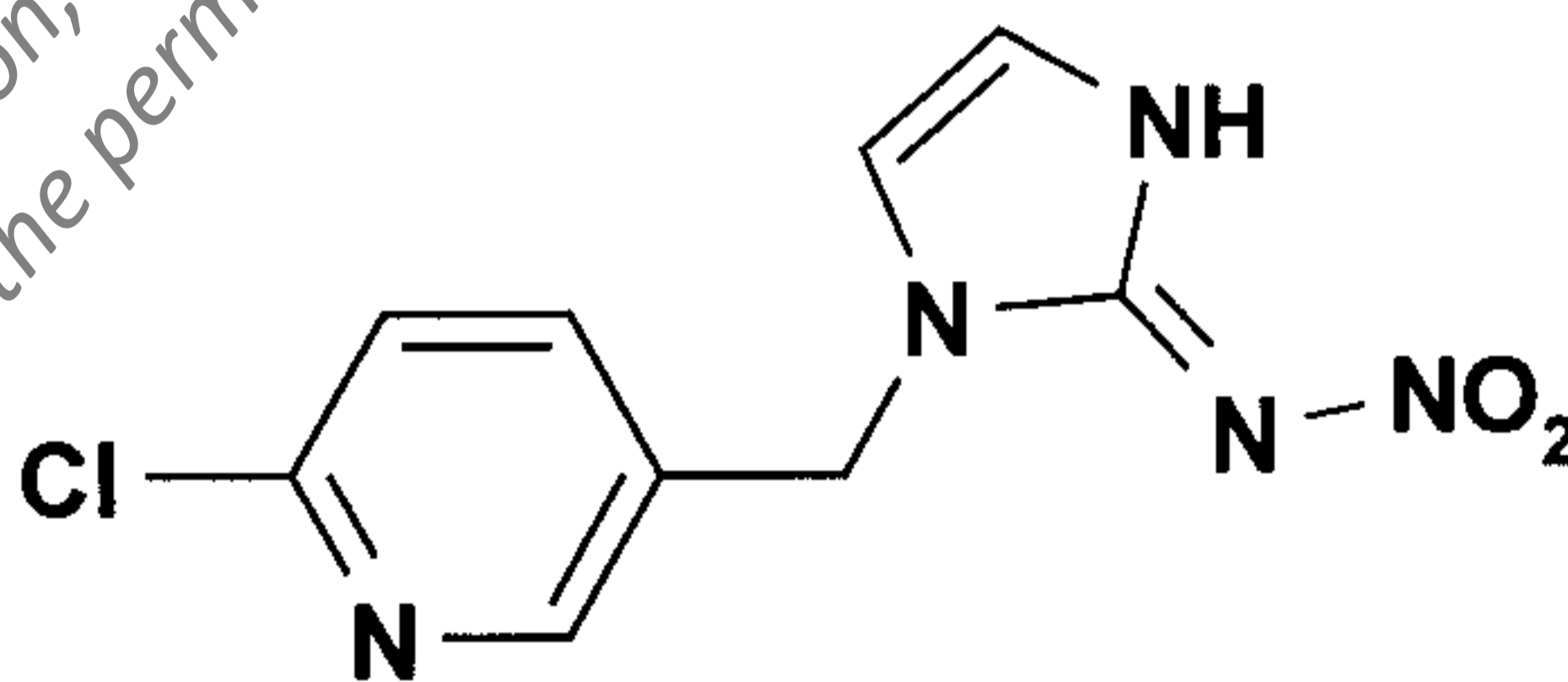
Structural formula:



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. 10 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 CH₂Cl₂. 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
	0.1 min	Flow to Waste
	4 min	Flow to MS
	10 min	Flow to Waste
	10.2 min.	11.1 % B
	10.3 min	90 % B
	11 min	90 % To Waste
	15.5 min	90 % B
	15.6 min	11.1 % B
	18.8 min	11.1% B TO MS
	19 min	Stoptime

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

Dit document is geen eigendom van het Ctgb en wordt beschouwd als openbaar. Het maakt op grond van een wettelijke verplichting tot openbaarmaking.
 Op dit document kunnen rechten van derden bestaan. Het gebruik van dit document is beperkt tot de informatie die nodig is voor de uitvoering van de
 Publicatie, verspreiding, vermenigvuldiging, commercieel gebruik en gebruik van dit document kan derhalve verboden zijn en een inbreuk op de rechten van de
 This document is not the property of the Ctgb and only provided for mandatory freedom of information requirements.
 The document may be subject to rights such as intellectual property and copy rights of third parties.
 Furthermore, this document may fall under regulatory data protection regime.
 Consequently, any publication, distribution, reproduction or publishing and any commercial exploitation and use of this document or its contents
 without the permission of the owner of this document may therefore be prohibited and violate the rights of its owner.

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 width 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^{18} atoms/cm². 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 Quadrupole 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 nitrogen 5.0)
Curtain gas: 1.44 L/min (liquid nitrogen 5.0)
Collision gas: 0.87 L/min (liquid nitrogen 5.0)
Turbo gas: 7 L/min (liquid nitrogen 5.0)

Scan Type: MRM (Multiple Reaction Monitoring Mode)
Polarity: Positive
Acquisition 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	-23
Imidacloprid (Cl 35)	256	209	250	-23
Hydroxy-Metabolite (Cl 37)#	274	191	250	-25
Hydroxy-Metabolite (Cl 35)	272	191	250	-25
Olefin-Metabolite (Cl 35)#	254	236	250	-13
Olefin-Metabolite (Cl 35)	254	205	250	-21

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