

Final Report

Study Title

Determination of residue levels of imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine in bee-relevant matrices of winter rape in a cereal succeeding crop scenario at Bayer CropScience AG experimental farm "Höfchen", Germany

Test Item

Fuberidazole + imazalil + imidacloprid + triadimenol FS 145.2 (7.2+8+70+60) G

Test Item (short)

FBZ + ILL + IMD + TDL FS 145.2 (7.2+8+70+60) G

Test Guideline

Special design study – no guideline available

Study Director

[REDACTED]

Authors

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Study Completion Date

April 27, 2011

Test Facility

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GLP Study Number

E 319 3387-4

Lynx Activity ID

EBNTL006



M-406083-01-1

1 DATA CONFIDENTIALITY STATEMENT

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2 GOOD LABORATORY PRACTICE STATEMENT

This study was conducted in compliance with the Principles of Good Laboratory Practice (Chemikaliengesetz, current version of Annex 1 and the current OECD Principles of Good Laboratory Practice (GLP), dated 1997-11-26 [C(97) 187/Final]). The test facility "BCS-Development - Environmental Safety - Ecotoxicology" and the test site "BCS-Development - Human Safety - Residue Analytics" had been inspected and certified as working in compliance with the Principles of Good Laboratory Practice by the competent authorities (GLP certificate reference: II A 5 – 31.11.60.05, April 23, 2009 and II A 5 – 31.11.91.02, June 16, 2009). The seed dressing procedure and the recording of the climatic conditions were not part of GLP.

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Date: 2011-04-27

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Date: 2011-04-27

Representative of the sponsor
and Head of test Facility:

Date: 2011-04-27

3 CERTIFICATION OF AUTHENTICITY



2011-04-27
Date

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4 QUALITY ASSURANCE STATEMENT

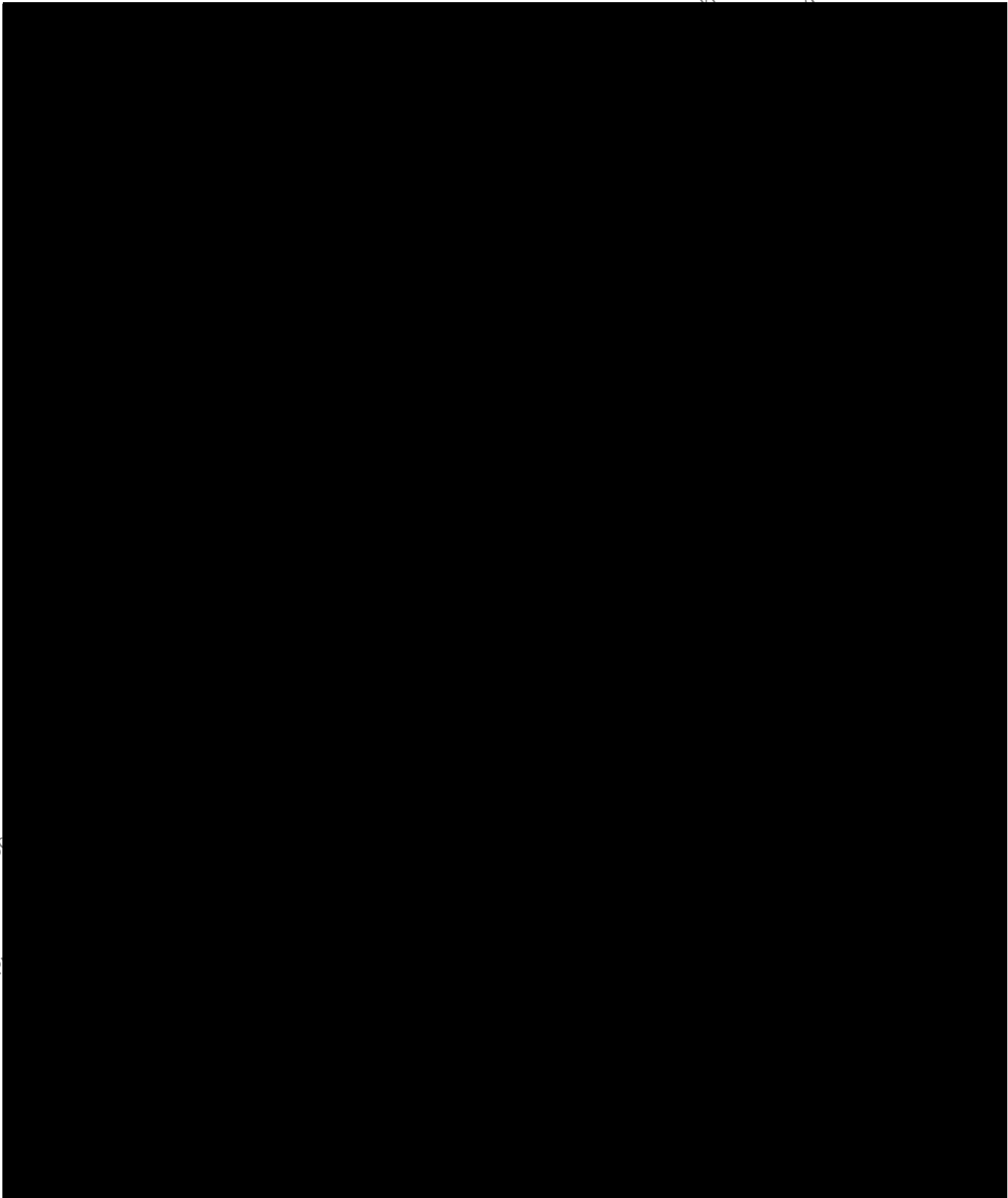


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5 SUMMARY

Report: [REDACTED] (2011):

Determination of residue levels of imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine in bee relevant matrices of winter rape in a cereal succeeding crop scenario at Bayer CropScience AG experimental farm "Höfchen", Germany, Bayer CropScience AG, unpublished report No.: E 319 3387-4, Date: April 27, 2011

Guidelines: No standard guideline available, test was particularly designed for the purpose of this study.

GLP: yes (certified laboratory)

Experimental starting/completion date: October 17, 2007 – August 24, 2009

Material and methods:

The imidacloprid containing test item (mixture of imidacloprid and fungicides), used for the purpose of this study, was fuberidazol + imazalil + imidacloprid + triadimenol FS 145.2 (7.2+8+70+60) G, TOX-No. of test item: 08068-00; analysed content of imidacloprid: 72.3 g a.s./L; density: 1.081 g/mL. In addition: imidacloprid-treated winter wheat seeds of the variety "Dekan"; dressed with the above mentioned test item (TOX-No. of treated seeds: 08079-00; analysed content of imidacloprid: 70.75 g a.s. /100 kg seeds; imidacloprid-free dressed winter wheat seeds of the variety "Dekan" as well as imidacloprid-free dressed winter oil-seed rape (OSR) seeds of the variety "Adriana".

In autumn 2007 (19 October 2007), the imidacloprid-containing test item was applied and incorporated down to 20 cm soil depth on a fallow test plot (=treatment test plot) at a rate corresponding to nominally 126 g a.s. imidacloprid/ha to conservatively establish a long-term soil plateau concentration of imidacloprid, simulating the consecutive use of imidacloprid on the same field plot over several years. Incorporation was achieved by means of a power-harrow. On the same day, immediately after the establishment of the long-term soil plateau concentration of imidacloprid, imidacloprid-treated winter wheat seeds, dressed with test item (=treatment winter wheat seeds), were sown on the treatment test plot at a nominal sowing rate of 180 kg seeds/ha, corresponding to nominally 126 g a.s. imidacloprid/ha. On an equivalent control test plot, imidacloprid-free dressed wheat seeds (=control winter wheat seeds) of the same variety as the treatment seeds were sown at the same day (19 October 2007). These imidacloprid-free control winter wheat seeds received the same nominal loading of active fungicidal substances as the treatment seeds. The control seeds were sown on the control test plot also at a nominal sowing rate of 180 kg seeds/ha. On the control test plot, no plateau concentration has been established, and as such, no spray application was performed.

In late summer 2008 (21 August 2008), after harvesting of the winter wheat at 01 August 2008, winter OSR seeds with an imidacloprid-free seed coating (insecticidal seed coating: Elado® (= 400 g clothianidin a.s. /L + 80 g beta-cyfluthrin a.s./L) + fungicidal seed coating "Thiram" (= 700 g thiram a.s./L)) were sown on the treatment test plot and the control test plot, respectively. No further crop was sown during the intervening period after harvesting of winter wheat and sowing of winter OSR seeds, as typical for commercial agricultural practice.

Seven days before foraging honeybees were exposed to the flowering winter OSR crop under confined conditions, one gauze tunnel (approximately 50 m² surface areas) was set up on the treatment test plot and the control test plot, respectively (13 April 2009). Thereafter, one honeybee colony with about 3000 bees (*Apis mellifera carnica*) was installed inside the tunnel on the treatment test plot and the control test plot, respectively (20 April 2009). During the flowering period of winter OSR, nectar- and pollen foraging honeybees were manually collected inside the tunnels and stored deep frozen. Afterwards, the frozen honeybees were worked up by separating pollen loads from the legs of the pollen forager bees and by extracting bee-collected nectar by puncturing the honey bulbs of the nectar forager bees with an ultra-fine syringe. Thereafter, the extracted pollen and nectar was analysed to determine residue levels of imidacloprid and its metabolites imidacloprid-monohydroxy and imidacloprid-olefine.

Findings:

Imidacloprid residues in soil

Directly after the application and incorporation of the test item into the upper 20 cm of the soil of the treatment test plot, the mean analysed imidacloprid concentration was 34.0 µg a.s./kg dry soil.

After a period of nearly 10 months, directly before sowing winter OSR seeds with an imidacloprid-free seed coating, the mean imidacloprid concentration on the treatment test plot decreased to 15.2 µg a.s./kg dry soil. The corresponding parallel soil residue analysis on the control test plot showed no residues of imidacloprid.

Analytical results for imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine in bee relevant matrices of winter OSR:

Sample Number	Sample Name	Sample Material	Treatment / Control Test Plot [T/C]	Residue [mg/kg]		
				Imidacloprid	imidacloprid-monohydroxy	imidacloprid-olefine
002	Pollen C2	Pollen	C	< LOD	0.004	< LOD
004	Pollen C4		C	< LOD	< LOD	< LOD
006	Pollen C6		C	< LOD	< LOD	< LOD
008	Pollen C8		C	< LOD	< LOD	< LOD
001	Pollen T1		T	0.0003	< LOD	< LOD
003	Pollen T3		T	< LOD	< LOD	< LOD
005	Pollen T5		T	0.0003	< LOD	< LOD
007	Pollen T7		T	< LOD	< LOD	< LOD
002	Nectar C2	Nectar	C	< LOD	< LOD	< LOD
004	Nectar C4		C	< LOD	< LOD	< LOD
006	Nectar C6		C	< LOD	< LOD	< LOD
008	Nectar C8		C	< LOD	< LOD	< LOD
001	Nectar T1		T	< LOD	< LOD	< LOD
003	Nectar T3		T	< LOD	< LOD	< LOD
005	Nectar T5		T	< LOD	< LOD	< LOD
007	Nectar T7		T	< LOD	< LOD	< LOD

Limit of quantitation (LOQ) for imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine = 0.001 mg/kg,
Limit of detection (LOD) for imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine = 0.0003 mg/kg

Conclusion:

Under still unrealistic worst case conditions (long-term imidacloprid plateau concentration conservatively simulated by fresh application and incorporation of imidacloprid into the soil at the day of sowing imidacloprid-dressed winter wheat, followed by winter OSR as a succeeding crop), residues of imidacloprid in OSR-pollen and OSR-nectar collected on the imidacloprid treatment test plot were always below the limit of quantitation (LOQ).

The imidacloprid concentration in two pollen samples from the treatment test plot matched the limit of detection (LOD) of 0.0003 mg a.s./kg, respectively, and in two pollen samples from the treatment test plot the imidacloprid concentration was < LOD, respectively. The imidacloprid-monohydroxy and imidacloprid-olefine concentration of all pollen samples from the treatment test plot was < LOD. The imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine concentration of all nectar samples from the treatment test plot was < LOD.

The residue finding of imidacloprid-monohydroxy in one of the pollen samples collected on the control test plot ("Pollen C2") is suspected to result from a contamination in the analytical laboratory, as neither parent imidacloprid nor imidacloprid-olefine was detected in this particular sample.

6 INTRODUCTION AND PURPOSE OF THE STUDY

This study aims to examine the exposure of honeybees to imidacloprid and its metabolites imidacloprid-monohydroxy and imidacloprid-olefine by potential uptake of soil residues of imidacloprid into winter rape nectar and pollen in a crop scenario.

7 RESPONSIBILITIES

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8 TIME SCHEDULE

Approval of study plan by the study director	18 September 2007
Start of field part	17 October 2007
End of field part	03 May 2009
Start of soil residue analytical part	19 October 2007
End of soil residue analytical part	13 March 2009
Start of bee matrix residue analytical part	21 April 2009
End of bee matrix residue analysis part	24 August 2009
Date of final report	27 April 2011

9 ARCHIVING

All raw data pertaining to this study and the original final report are stored in the central GLP archive of Bayer CropScience AG, Alfred-Nobel-Str. 50, D-40789 Monheim am Rhein for as long as required by GLP principles.

Reserve samples of the test item are stored in the pertinent reserve sample archive of Bayer CropScience AG Development, Formulation Technology, Analysis & Services, Alfred-Nobel-Strasse 50, D-40789 Monheim am Rhein, Germany. The test items are stored as long as their quality still guarantees an evaluation.

10 EXPERIMENTAL PART

10.1 Test Items and Treated Seeds

The test item (see 10.1.1) was used for both, application and incorporation on the treatment field plot to establish a long-term soil plateau concentration of imidacloprid (application of 1743 mL test item/ha via 300 L spray solution/ha, corresponding to 126 g imidacloprid a.s./ha) and for the preparation of the treatment winter wheat seeds (see 10.1.2). The imidacloprid-free control winter wheat seeds (see 10.1.3) received the same nominal loading of active fungicidal substances as the treatment winter wheat seeds, except for imidacloprid. Both, the treatment and the control winter wheat seeds were sown on the respective test plots at a sowing rate of 180 kg seeds/ha.

10.1.1 Test Item

Common name(s):	a) Fuberidazole b) Imazalil c) Imidacloprid d) Triadimenol
Trade name:	Manta Plus FS 145.2 (= "Baytan U + Gaucho")
Active substances (a.s.)	a) W VII/117 b) R-23979 c) NTN 33893 d) KWG 0519
Formulation:	FS 145.2 (7.2+8+70+60) G
CAS No.:	a) 3878-19-1 b) 35554-44-0 c) 138261-41-3 d) 55219-65-3
Material No.:	05268206
Specification No.	10200007547
Batch No.	2007-009957
TOX No.	08068-00
Density:	1.081 g/mL
Content of a.s. (analytical):	a) 7.5 g/L (0.693% w/w) b) 8.7 g/L (0.803% w/w) c) 72.3 g/L (6.69% w/w) d) 60.9 g/L (5.63% w/w)
Analytical procedure:	a) W VII/117/2001-0043901-97/GLC, ISTD/AZ 11287 b) R-23979/2001-0043901-97/GLC, ISTD/AZ 14073 c) NTN 33893/2001-0002502-97/HPLC, ESTD/AZ 13319 d) KWG 0519/2001-0043901-97/GLC, ISTD/AZ 13279
Date of analysis:	25 September 2007
Expiry date:	25 September 2009
Storage conditions:	25 ±5°C, storage conditions from +2°C to +30°C are also acceptable
Indication:	Insecticide and fungicide seed treatment
Physical appearance:	Red suspension

10.1.2 Treatment winter wheat seeds (sown on the treatment test plot)

Species:	Winter wheat
Variety:	Dekan (authentication No.: D/BN 43174162)
Origin of native seeds:	KWS Lochow GmbH, 29296 Bergen, Germany
Thousand kernel weight:	47.8 g
Germination capacity:	99%
Test item used for seed treatment: (TOX-No. 08068-00):	Fuberidazole + imazalil + imidacloprid + triadimenol FS 145.2 (7.2+8+70+60) G (= Manta Plus FS 145.2)
Nominal seed dressing rate:	1 L test item/100 kg seeds (=70 g a.s. imidacloprid/100 kg seeds)
Measured seed dressing rate:	70.75 g / 100 kg seeds (101.1% of nominal)
TOX-No. of treated seeds:	08079-00
Analytical procedure:	HPLC, ESTD
Date of analysis:	12 October 2007
Expiry date:	12 April 2008
Seed treatment performed by:	Bayer Crop Science AG, Portfolio Management Seed Treatment Application Centre 40789 Monheim, Germany

The seed dressing procedure was not conducted under GLP.

10.1.3 Control winter wheat seeds (sown on the control test plot)

Species:	Winter wheat
Variety:	Dekan (authentication No.: D/BN 43174162)
Origin of native seeds:	KWS Lochow GmbH, 29296 Bergen, Germany
Thousand kernel weight:	47.8 g
Germination capacity:	99%
Fungicide product used for seed treatment: (TOX-No. 07812-00):	Fuberidazol + imazalil + triadimenol FS 94 (9+10+75) G (="Baytan U)
Fungicide dressing rate (nominal):	0.8 L product/100 kg seeds
Seed treatment performed by:	Bayer Crop Science AG, Portfolio Management Seed Treatment Application Centre 40789 Monheim, Germany

The seed dressing procedure was not conducted under GLP.

10.1.4 Imidacloprid-free dressed winter oil-seed rape (OSR) seeds (sown on the treatment and the control test plot, respectively)

Species:	Winter OSR
Variety:	Adriana (authentication No.: 082147)
Origin of native seeds:	Limagrain GmbH, 31234 Edemissen, Germany
Thousand kernel weight:	6.03 g
Germination capacity:	95%
Standard fungicide dressing rate (nominal):	Commercial Thiram SC 700 (= nominal 700 g thiram/L) 5.71 mL commercial product/kg seeds
Standard insecticide dressing rate (nominal):	Commercial Elado FS 480 (= nominal 400 g clothianidin/L + 80 g beta-cyfluthrin/L) 25 mL commercial product/kg seeds
Seed treatment performed by:	Bayer Crop Science AG, Portfolio Management Seed Treatment Application Centre 40789, Monheim, Germany

The seed dressing procedure was not conducted under GLP.

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10.2 Study Conduction of the Field Part

10.2.1 Test Location, Test Plots and Soil Characterization

The study was conducted in Germany, North Rhine-Westphalia, close to Burscheid at the Bayer Experimental Farm "Höfchen" at the field area "Am Hohensen", field number 410. The size of the field area was about 2116 m², where two test plots (treatment and control) were set up separately in approximately 10 meter distance to avoid possible cross-contamination. Soil parameters such as particle size distribution according to DIN 19682, pH value, water holding capacity and organic carbon content were determined in BCS-D-EnSa-ETX and the "LUFA Speyer" (Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer).

Size control plot:	828 m ²
Size treatment plot:	828 m ²
Control and treatment plot:	C _{org.} -content: 1.29%, Max. water holding capacity: 50.08 g H ₂ O/100 g dry soil, pH value: 6.48 sand 3.4% wt, silt 81.2% wt, clay 15.4% wt = 'lehmiger Schluff' ("loamy silt) according to DIN 19682

10.2.2 Test Organism

None, the study was focused on determination of residues from winter OSR pollen and nectar, not on effects on organisms.

10.2.3 Establishing the Soil Plateau Concentration

The plateau concentration of imidacloprid in soil was calculated by using FOCUS-PEARL, considering the consecutive use of imidacloprid according to the following three-year cropping cycle: 117 g imidacloprid a.s./ha via dressed sugar beet pills, followed by 126 g imidacloprid a.s./ha via dressed cereals, followed by 126 g imidacloprid a.s./ha via dressed cereals. The consecutive iteration of the above outlined three-year cropping cycle results into a calculated long-term plateau concentration of 38 µg imidacloprid a.s./kg soil for the upper 20 cm soil layer. In order to conservatively establish this long-term soil plateau concentration, the test item was freshly applied and incorporated into the upper 20 cm soil layer of the test item treatment plot before sowing of the treatment seeds on the same day (19 October 2007). Thus, a soil concentration of 38 µg a.s./kg soil would represent worst-case conditions for the selected succeeding imidacloprid-seed-treated winter wheat - imidacloprid-free dressed winter OSR cultivation scenario. The treatment plot was sprayed with a sprayer type "Amazone US 404T". The working width of the first lane was 12 m and the working width of the second lane was 6 m (half numbers of the nozzles were closed). The working pressure of the sprayer was 2 bar and the driving speed was 6 km/h. After the application, the applied product was incorporated into 20 cm soil depth with a power harrow.

The control plot remained untreated.

10.2.4 Sowing of Winter Wheat on the Study Plots

The sowing of the treatment winter wheat seeds (see 10.1.2) on the treatment plot was performed on 19 October 2007, after the establishment of the soil plateau concentration (see 10.2.3, above). The sowing rate was 180 kg seeds/ha. The machinery employed for the sowing operation was an "Amazone AD 302" sowing machine.

The sowing of the control winter wheat seeds (see 10.1.3) on the control plot was performed on the same day as the sowing of the treatment winter wheat seeds, by employing the identical sowing rate and by using the same sowing equipment.

The sowing operation was performed as typical for commercial agricultural practice on both study plots. On 01 August 2008, the winter wheat was harvested.

On 21 August 2008, imidacloprid-free dressed winter OSR seeds (see 10.1.4) were sown on the treatment test plot and the untreated control test plot, respectively. The nominal winter OSR seed sowing rate on both study plots was 600,000 seeds/ha. The machinery used for OSR sowing was an "Amazone AD 302" sowing machine. The sowing operation was performed as typical for commercial agricultural practice on both study plots.

No further crop was sown during the intervening period after harvesting of winter wheat on 01 August 2008 and sowing of winter OSR seeds on 21 August 2008, as typical for commercial agricultural practice.

The size of the treatment test plot and the control test plot was about 828 m², respectively. The plots were about 10 meters separated from each other to avoid cross-contamination.

10.2.5 Soil Sampling and Imidacloprid Analysis in Soil

On 19 October 2007, immediately after spray application and incorporation of the test item into the upper 20 cm soil layer of the treatment test plot (before sowing of the treatment winter wheat seeds), soil samples from the treatment plot were taken to quantify the established soil plateau concentration of imidacloprid. Fifteen soil samples were taken down to a depth of 30 cm (for technical reasons) with a hydraulic soil sampling machine (Ø 49 mm).

A second soil sampling was conducted on the treatment test plot on 21 August 2008, immediately before drilling of the imidacloprid-free dressed winter OSR seeds. Fifteen soil samples per plot were taken by means of a hydraulic soil sampling machine (Ø 49 mm) down to a depth of 30 cm (for technical reasons).

From all soil samples taken by means of the hydraulic sampling device, only the upper 20 cm were worked up, the lower 10 cm were cut off and discarded.

Finally, the 15 soil samples of each sampling date were pooled to one single composite sample for residue analysis. All soil samples were worked up for residue analysis by BCS-D-HS-RA.

The soil samples were stored deep frozen until work-up and analysis in the Laboratory of Mr. Th. Freitag (BCS-D-HS-RA) according to the method 00790/M001.

A detailed analytical phase report (soil analysis) is attached in Appendix 5 and the results are summarized in Table 1.

10.2.6 Honeybee Colonies

With the beginning of the flowering period of the winter OSR, a single gauze tunnel of about 50 m² ground area was set up on each study plot, seven days before the honeybee colonies were established inside the tunnels. One honeybee colony was used per gauze tunnel. The honeybee colonies were established inside the tunnels on 20 April 2009 and were kept under confinement until the end of the flowering period (03 May 2009). Each honeybee colony contained combs with approximately 3000 bees (*Apis mellifera carnica*). The colonies were provided by a local beekeeper, [REDACTED]

10.2.7 Sampling of Foraging Honeybees

Between 21 April 2009 and 02 May 2009, on four different sampling days during the flowering period of the winter OSR, foraging honeybees were collected from the hive entrance. The bees were collected manually by using tweezers and a small collecting vessel, covered with crosswise incised gauze. The bees were killed immediately by means of dry ice. Different sets of sampling vessels were used for the control and the treatment test plot in order to prevent cross-contamination. At each sampling day, as much honeybees as available were collected. After each sampling session, the bees were directly transferred to an appropriate container and stored deep-frozen until work-up for analysis. Each sample taken from the study plots was identified by the GLP study number, the plot identification, the sample number and the sampling date.

10.2.8 Pollen and Nectar Extraction from the Sampled Bees

The sampled bees were separated into pollen- and nectar carrying honeybees (i.e. pollen forager bees and nectar forager bees). The pollen and nectar loads were extracted at room temperature. For each extraction session, a small proportion of pollen- or nectar forager samples were removed from the deep freezer. In order to prevent cross-contamination, all control samples were worked up first. For all working steps, different material for control and treatment bees was used. Additionally, laboratory equipment and instruments were cleaned prior and between sample extractions using alcohol-containing disinfection liquid. Bees were processed promptly and never left unattended. After each pollen and nectar extraction session, all samples were re-frozen.

During each extraction session, bees with pollen loads (pollen forager bees) were placed into a fresh Petri dish. Individual bees were lifted from the dish using tweezers. If present, pollen was removed from the *tibia* using a sterile preparation needle. For each sampling day and study plot, the collected pollen loads were transferred into a sterile vial which was labelled appropriately.

The nectar was removed directly from the honey bulb of individual nectar forager bees by introducing a 0.6 x 30 mm ultra-fine hypodermic canula (attached to a 1 mL syringe) diagonal from the top into the abdomen of the bee. The syringe was fixed within an apparatus, which is normally used for the artificial insemination of honeybee queens. The apparatus was adapted to the needs of nectar extraction.

The nectar foragers were placed into a specially manufactured plastic device which was designed to hold 10 bees in individual grooves. After the extraction, the nectar was transferred into an appropriately labelled sterile vial.

10.2.9 Climatic Conditions

The climatic conditions on the test field (such as temperature, humidity and rainfall) were obtained from the nearby weather station at the Bayer CropScience AG Experimental Farm "Höfchen". The temperature of the freezer, where the samples were stored until the transfer for residue analysis, was recorded by a min./max. thermometer.

The following climatic conditions were recorded on 19 October 2007, the day of establishing the imidacloprid soil plateau concentration on the treatment plot and the sowing of the treatment- and the control winter wheat seeds.

- Average temperature (soil 0 cm): 7.5°C
- Sum precipitation: 0.4 mm
- Average relative humidity: 78%
- Sum sunshine: 0.8 hours/day

The climatic conditions on the day of winter OSR sowing (21 August 2008) were recorded as follows:

- Average temperature (soil 0 cm): 17.1°C
- Sum precipitation: 0.3 mm
- Average relative humidity: 76%
- Sum sunshine: 3.7 hours/day

In April and May 2009, when the bee colonies were established inside the gauze tunnels and the bee sampling was conducted, the following climatic conditions were recorded:

Date	Mean Air Temperature (2 m above ground)	Humidity	Precipitation	Sunshine
[YYYY-MM-DD]	[°C]	[%]	[mm]	[hours]
2009-04-20*	14.27	71	0.0	10.44
2009-04-21**	15.22	56	0.0	12.74
2009-04-22	11.19	71	0.0	9.34
2009-04-23	10.42	65	0.0	5.25
2009-04-24	12.52	61	0.0	12.20
2009-04-25	14.91	60	0.0	8.83
2009-04-26	15.76	68	9.7	8.64
2009-04-27	12.80	73	1.2	3.80
2009-04-28	9.80	92	4.4	0.00
2009-04-29**	11.00	72	0.1	6.79
2009-04-30**	12.34	73	0.0	2.15
2009-05-01	15.63	70	0.1	10.19
2009-05-02**	14.74	68	0.0	11.38
2009-05-03***	12.03	76	0.0	2.16

* Honeybee colonies were established in the tunnels

** Sampling days of foraging bees with pollen and nectar

*** Relocation of the bee colonies from the tunnels

10.3 Nectar and Pollen Analysis

The pollen and nectar samples were analyzed in the laboratory of [REDACTED] Bayer CropScience AG, Institute for Human Safety, Residue Analytics (BCS-D-HS-RA) using the following method: [REDACTED] (02 November 2006): Modification M002 of the Residue Analytical Method No. 00537 for the determination of residues of imidacloprid and its metabolites NTN33893-5-hydroxy (= imidacloprid-monohydroxy) and NTN33893-olefine (= imidacloprid-olefine) in Nectar (Honey) and Pollen by HPLC with electro spray MS/MS-detection. (Method No. 00537/M002, Report No. MR-06/144). A detailed report of nectar and pollen analysis is attached in Appendix 6.

11 RESULTS

11.1 Imidacloprid Concentration in Soil

Directly after the application and incorporation of the test item into the upper 20 cm of the soil of the treatment test plot the mean analysed imidacloprid concentration was 34.0 µg a.s./kg dry soil.

After a period of nearly 10 months, directly before sowing winter OSR seeds with an imidacloprid-free seed coating, the mean imidacloprid concentration of the treatment test plot decreased to 15.2 µg a.s./kg dry soil. The corresponding parallel soil residue analysis on the control test plot showed no residues of imidacloprid.

A detailed report of the soil analysis is attached in Appendix 5 and the results are summarized in Table 1.

11.2 Residues in Winter OSR Nectar and Pollen

Between 21 April 2009 and 02 May 2009 (=sampling period), on four different sampling days, in total approx. 3804 foraging bees were collected (approx. 1961 from the control test plot, approx. 1843 from the treatment test plot).

The limit of quantitation (LOQ) for all analytes in nectar and pollen was 0.001 mg/kg. The limit of detection (LOD) for all analytes in nectar and pollen was 0.0003 mg/kg.

The honeybees from the control test plot were separated into 314 bees with pollen load (pollen forager bees) and approx. 1647 nectar forager bees. Throughout the sampling period, in total 1.9708 g pollen was sampled from the pollen forager bees on the control test plot (on average, 6.3 mg pollen/bee). From the total number of approx. 1647 nectar forager bees collected on the control test plot, the nectar of 423 bees was extracted from the bees' honey bulbs for the analytical determination of residues of imidacloprid and its metabolites. The remaining nectar forager bees were stored in the deep-freezer until the end of the study and were discarded after the finalization of the study. Throughout the sampling period, in total 7.2273 g nectar was extracted (on average, 17.1 mg nectar/bee).

The imidacloprid and imidacloprid-olefine concentration in all pollen samples from the control test plot was < LOD (limit of detection; 0.0003 mg/kg). The imidacloprid-monohydroxy concentration in three of four pollen samples from the control test plot was < LOD and in a single pollen sample from the control test plot the concentration was 0.004 mg/kg. The imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine concentration in all nectar samples from the control test plot was < LOD.

The residue finding of imidacloprid-monohydroxy in one of the pollen samples collected on the control test plot ("Pollen C2") is suspected to result from a contamination in the analytical laboratory, as neither parent imidacloprid nor imidacloprid-olefine was detected in this particular sample.

The honeybees from the treatment test plot were separated into 271 bees with pollen load (pollen forager bees) and approx. 1572 nectar forager bees. Throughout the sampling period, in total 1.8821 g pollen was sampled from the pollen forager bees on the treatment test plot (on average, 6.9 mg pollen/bee). From the total number of approx. 1572 nectar forager bees collected on the treatment test plot, the nectar of 535 bees was extracted from the bees' honey bulbs for the analytical determination of residues of imidacloprid and its metabolites. The remaining nectar forager bees were stored in the deep-freezer until the end of the study and were discarded after the finalization of the study. Throughout the sampling period, in total 6.3591 g nectar was extracted (on average, 11.9 mg nectar/bee).

The imidacloprid concentration in two pollen samples from the treatment test plot matched the limit of detection (LOD) of 0.0003 mg a.s./kg, respectively, and in two pollen samples from the treatment test plot the imidacloprid concentration was < LOD, respectively. The imidacloprid-monohydroxy and imidacloprid-olefine concentration of all pollen samples from the treatment test plot was < LOD. The imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine concentration of all nectar samples from the treatment test plot was < LOD.

The analytical results for imidacloprid and its metabolites imidacloprid-monohydroxy and imidacloprid-olefine in the nectar and pollen samples are displayed in Table 2. A detailed analytical phase report, including a description of the employed methods, the individual findings of the nectar and pollen analysis as well as representative chromatograms, is included in Appendix 6.

12 TABLES

Table 1 Concentration of Imidacloprid in the Upper 20 cm of Soil of the Treatment Test Plot

Sampling moment	Sampling date	Study plot	Mean measured concentration of imidacloprid [$\mu\text{g}/\text{kg}$ dry soil]
Directly after spray application and incorporation	19 October 2007	Treatment	34.0
Directly before sowing of winter OSR	21 August 2008	Treatment	15.2
Directly before sowing of winter OSR	21 August 2008	Control	< LOD

Limit of quantitation (LOQ) of the method is $5 \mu\text{g}/\text{kg}$ soil for Imidacloprid

Limit of detection (LOD) = $2 \mu\text{g}/\text{kg}$ soil for Imidacloprid

Table 2 Imidacloprid Residues in Winter OSR Nectar and Pollen

Sample Number	Sample Name	Sampling Date	Sample Weight [g]	Treatment / Control Test Plot [T/C]	Residue [mg/kg]		
					Imidacloprid	NTN33893-5-hydroxy	NTN33893-olefine
002	Pollen C2	2009-04-21	0.0451	C	< LOD	0.004	< LOD
004	Pollen C4	2009-04-29	0.5655	C	< LOD	< LOD	< LOD
006	Pollen C6	2009-04-30	0.8363	C	< LOD	< LOD	< LOD
008	Pollen C8	2009-05-02	0.5239	C	< LOD	< LOD	< LOD
001	Pollen T1	2009-04-21	0.1061	T	0.0003	< LOD	< LOD
003	Pollen T3	2009-04-29	0.4394	T	< LOD	< LOD	< LOD
005	Pollen T5	2009-04-30	0.8202	T	0.0003	< LOD	< LOD
007	Pollen T7	2009-05-02	0.5164	T	< LOD	< LOD	< LOD
002	Nectar C2	2009-04-21	1.7717	C	< LOD	< LOD	< LOD
004	Nectar C4	2009-04-29	1.4733	C	< LOD	< LOD	< LOD
006	Nectar C6	2009-04-30	1.9816	C	< LOD	< LOD	< LOD
008	Nectar C8	2009-05-02	2.0007	C	< LOD	< LOD	< LOD
001	Nectar T1	2009-04-21	1.6078	T	< LOD	< LOD	< LOD
003	Nectar T3	2009-04-29	1.4880	T	< LOD	< LOD	< LOD
005	Nectar T5	2009-04-30	1.6905	T	< LOD	< LOD	< LOD
007	Nectar T7	2009-05-02	1.5728	T	< LOD	< LOD	< LOD

Limit of quantitation (LOQ) for imidacloprid, NTN33893-5-hydroxy and NTN33893-olefine = 0.001 mg/kg

Limit of detection (LOD) for imidacloprid, NTN33893-5-hydroxy and NTN33893-olefine = 0.0003 mg/kg

Table 3 Climatic Conditions during the Year 2007

Metrological station Höfchen	Temperature Soil 0 cm	Humidity	Precipitation	Sunshine
	[°C]	[%]	[mm]	[hours]
▲ Maxima	19.02	90		
∅ Mean	11.17	80		
▼ Minima	1.89	61		
∑ Sum			1066.5	1456.65
Month	Temperature Soil 0 cm	Humidity	Precipitation	Sunshine
[YYYY-MM]	[°C]	[%]	[mm]	[hours]
2007-01	4.48	84	88.8	19.82
2007-02	4.72	90	55.1	42.09
2007-03	6.65	79	62.4	142.56
2007-04	15.08	61	1.5	299.87
2007-05	16.52	77	116.5	167.85
2007-06	17.88	81	98.3	131.70
2007-07	18.69	79	122.7	156.28
2007-08	19.02	76	213.7	173.21
2007-09	14.12	78	71.4	106.20
2007-10*	10.32	82	41.6	132.49
2007-11	4.64	85	95.9	29.01
2007-12	1.89	84	98.6	55.56

* Spray application and sowing of winter wheat

Table 4 Climatic Conditions during October 2007

Metrological station Höfchen	Temperature Soil 0 cm [°C]	Humidity [%]	Precipitation [mm]	Sunshine [hours]
▲ Maxima	15.97	94		
∅ Mean	10.32	82		
▼ Minima	4.73	72		
Σ Sum			41.6	132.49
Date [YYYY-MM-DD]	Temperature Soil 0 cm [°C]	Humidity [%]	Precipitation [mm]	Sunshine [hours]
2007-10-01	13.30	85	5.6	1.30
2007-10-02	14.41	94	0.0	0.05
2007-10-03	15.97	90	4.6	1.96
2007-10-04	15.65	84	0.1	3.35
2007-10-05	12.64	82	0.1	5.21
2007-10-06	12.95	77	0.0	10.55
2007-10-07	13.01	76	0.0	10.51
2007-10-08	12.12	81	0.1	7.54
2007-10-09	12.58	85	0.0	5.48
2007-10-10	13.43	78	0.0	6.96
2007-10-11	12.50	77	0.0	7.83
2007-10-12	11.44	86	0.0	0.00
2007-10-13	13.57	76	0.0	9.05
2007-10-14	11.02	73	0.0	10.40
2007-10-15	12.18	72	0.0	9.85
2007-10-16	13.68	72	0.0	3.04
2007-10-17	11.11	80	2.1	0.52
2007-10-18	8.79	74	0.0	3.50
2007-10-19*	7.48	78	0.4	0.75
2007-10-20	6.73	74	0.0	7.72
2007-10-21	6.27	89	1.4	0.00
2007-10-22	5.06	82	0.0	9.45
2007-10-23	4.73	78	0.0	9.65
2007-10-24	5.77	84	0.0	1.21
2007-10-25	8.17	83	1.3	0.35
2007-10-26	7.74	82	0.0	0.00
2007-10-27	7.42	88	0.0	0.00
2007-10-28	8.01	82	0.0	0.81
2007-10-29	7.63	92	20.0	0.00
2007-10-30	8.42	84	5.8	2.40
2007-10-31	6.22	94	0.0	3.06

* Spray application and sowing of winter wheat

Table 5 Climatic Conditions during the Year 2008

Metrological station Höfchen	Temperature Soil 0 cm	Humidity	Precipitation	Sunshine
	[°C]	[%]	[mm]	[hours]
▲ Maxima	19.59	93	109.7	222.51
∅ Mean	10.70	81	76.1	118.09
▼ Minima	1.86	68	40.7	33.00
∑ Sum			912.8	1417.09
Month	Temperature Soil 0 cm	Humidity	Precipitation	Sunshine
[YYYY-MM]	[°C]	[%]	[mm]	[hours]
2008-01	3.60	82	69.0	42.42
2008-02	3.73	78	47.7	131.00
2008-03	4.77	83	92.3	72.90
2008-04	9.22	79	68.3	114.33
2008-05	17.78	68	40.7	222.51
2008-06	19.59	77	93.6	213.78
2008-07	19.46	81	108.9	177.99
2008-08*	18.19	80	93.9	133.55
2008-09	13.94	84	60.0	137.78
2008-10	10.09	92	109.7	80.61
2008-11	6.16	93	50.9	33.00
2008-12	1.86	79	77.8	57.24

* Harvesting of winter wheat and sowing of winter OSR

Table 6 Climatic Conditions during August 2008

Metrological station Höfchen	Temperature Soil 0 cm [°C]	Humidity [%]	Precipitation [mm]	Sunshine [hours]
▲ Maxima	22.53	94		
∅ Mean	18.19	80		
▼ Minima	15.79	69		
Σ Sum			93.9	133.55
Date [YYYY-MM-DD]	Temperature Soil 0 cm [°C]	Humidity [%]	Precipitation [mm]	Sunshine [hours]
2008-08-01*	22.53	73	0.7	3.91
2008-08-02	19.67	78	0.6	2.04
2008-08-03	19.64	80	2.4	1.10
2008-08-04	18.93	87	18.8	3.74
2008-08-05	18.43	81	0.0	3.61
2008-08-06	21.77	73	0.0	11.01
2008-08-07	21.29	76	3.8	6.99
2008-08-08	18.93	91	6.7	1.81
2008-08-09	19.08	78	0.0	6.79
2008-08-10	18.45	84	6.4	0.68
2008-08-11	19.27	77	0.4	6.64
2008-08-12	18.49	85	12.0	2.74
2008-08-13	16.88	71	2.6	4.76
2008-08-14	16.85	74	0.0	6.44
2008-08-15	17.01	75	0.0	8.82
2008-08-16	17.70	70	0.0	12.94
2008-08-17	18.16	69	0.1	6.11
2008-08-18	18.22	78	2.3	3.11
2008-08-19	18.10	76	0.0	2.62
2008-08-20	16.61	82	4.6	0.67
2008-08-21**	17.10	76	0.3	3.66
2008-08-22	17.20	94	8.9	0.02
2008-08-23	15.79	90	0.9	1.30
2008-08-24	16.53	84	0.8	3.72
2008-08-25	16.54	90	2.6	2.81
2008-08-26	16.72	84	0.0	0.04
2008-08-27	16.90	85	0.0	0.01
2008-08-28	16.79	90	0.0	0.00
2008-08-29	17.22	88	0.0	2.31
2008-08-30	18.15	78	0.1	11.87
2008-08-31	18.85	69	19.0	11.30

* Harvesting of winter wheat

** Sowing of winter OSR

Table 7 Climatic Conditions during April 2009

Metrological station Höfchen	Mean Temperature Air 2 m [°C]	Humidity [%]	Precipitation [mm]	Sunshine [hours]
▲ Maxima	18.46	96	19.9	12.74
∅ Mean	13.73	72	2.0	6.72
▼ Minima	9.64	56	0.0	0.00
Σ Sum			59.1	201.47
Date [YYYY-MM-DD]	Mean Temperature Air 2 m [°C]	Humidity [%]	Precipitation [mm]	Sunshine [hours]
2009-04-01	11.10	58	0.0	11.81
2009-04-02	14.61	57	0.0	11.86
2009-04-03	16.38	60	0.0	11.72
2009-04-04	13.76	80	0.0	3.78
2009-04-05	12.15	80	0.0	0.00
2009-04-06	14.99	68	0.0	8.34
2009-04-07	14.60	72	2.7	8.07
2009-04-08	11.88	82	2.7	4.27
2009-04-09	14.22	81	0.6	6.64
2009-04-10	18.46	56	0.0	11.21
2009-04-11	18.16	59	0.3	9.23
2009-04-12	16.15	80	12.8	4.65
2009-04-13	13.13	95	0.2	2.28
2009-04-14	14.90	80	0.0	5.65
2009-04-15	18.12	58	0.0	9.75
2009-04-16	15.24	72	4.7	2.51
2009-04-17	11.37	96	19.9	0.00
2009-04-18	9.64	87	0.0	0.85
2009-04-19	12.75	76	0.0	8.66
2009-04-20*	14.27	71	0.0	10.44
2009-04-21**	15.22	56	0.0	12.74
2009-04-22	11.19	71	0.0	9.34
2009-04-23	10.42	65	0.0	5.25
2009-04-24	12.52	61	0.0	12.20
2009-04-25	14.91	60	0.0	8.83
2009-04-26	15.76	68	9.7	8.64
2009-04-27	12.80	73	1.2	3.80
2009-04-28	9.80	92	4.4	0.00
2009-04-29**	11.00	72	0.1	6.79
2009-04-30**	12.34	73	0.0	2.15

* Honeybee colonies were established in the tunnels

** Sampling days of foraging bees with pollen and nectar

Table 8 Climatic Conditions during May 2009

Metrological station Höfchen	Mean Temperature Air 2 m [°C]	Humidity [%]	Precipitation [mm]	Sunshine [hours]
▲ Maxima	21.71	91	15.1	14.04
∅ Mean	14.71	71	1.8	6.23
▼ Minima	9.11	54	0.0	0.00
∑ Sum			56.0	193.06
Date [YYYY-MM-DD]	Mean Temperature Air 2 m [°C]	Humidity [%]	Precipitation [mm]	Sunshine [hours]
2009-05-01	15.63	70	0.1	10.19
2009-05-02**	14.74	68	0.0	11.38
2009-05-03***	12.03	76	0.0	2.16
2009-05-04	9.11	65	0.0	5.42
2009-05-05	9.42	91	15.1	0.00
2009-05-06	11.18	88	0.6	0.00
2009-05-07	14.85	70	0.0	9.41
2009-05-08	13.04	81	7.9	1.31
2009-05-09	11.59	80	2.1	4.43
2009-05-10	15.28	70	0.1	9.65
2009-05-11	13.06	85	5.4	0.00
2009-05-12	12.76	72	0.1	5.61
2009-05-13	14.95	68	0.0	2.68
2009-05-14	15.67	73	1.6	2.18
2009-05-15	14.35	88	10.4	0.84
2009-05-16	12.63	74	0.0	5.86
2009-05-17	14.14	76	5.0	1.54
2009-05-18	14.76	64	0.0	7.32
2009-05-19	15.10	71	1.9	8.74
2009-05-20	17.08	68	0.1	10.85
2009-05-21	17.50	65	0.1	4.40
2009-05-22	15.12	59	0.0	8.93
2009-05-23	16.30	58	0.1	11.73
2009-05-24	20.36	56	0.0	14.04
2009-05-25	21.71	57	0.3	9.27
2009-05-26	18.97	71	2.8	3.62
2009-05-27	12.23	71	0.0	4.58
2009-05-28	13.86	83	2.4	1.98
2009-05-29	14.90	54	0.0	13.91
2009-05-30	16.17	54	0.0	12.51
2009-05-31	18.17	61	0.0	8.54

** Sampling days of foraging bees with pollen and nectar

*** Removal of the bee colonies from the tunnels

Table 9 Climatic Conditions Refrigerator

The collected bees and the samples of nectar and pollen were stored deep-frozen in the refrigerator (Bayer inventory No. 598352).

The samples of nectar and pollen were transferred to the laboratory of [REDACTED] on 17 June 2009 for the analytical determination.

Time period	Temperature [°C]	
	Minimum	Maximum
May 2009 to June 2009	-21	-17

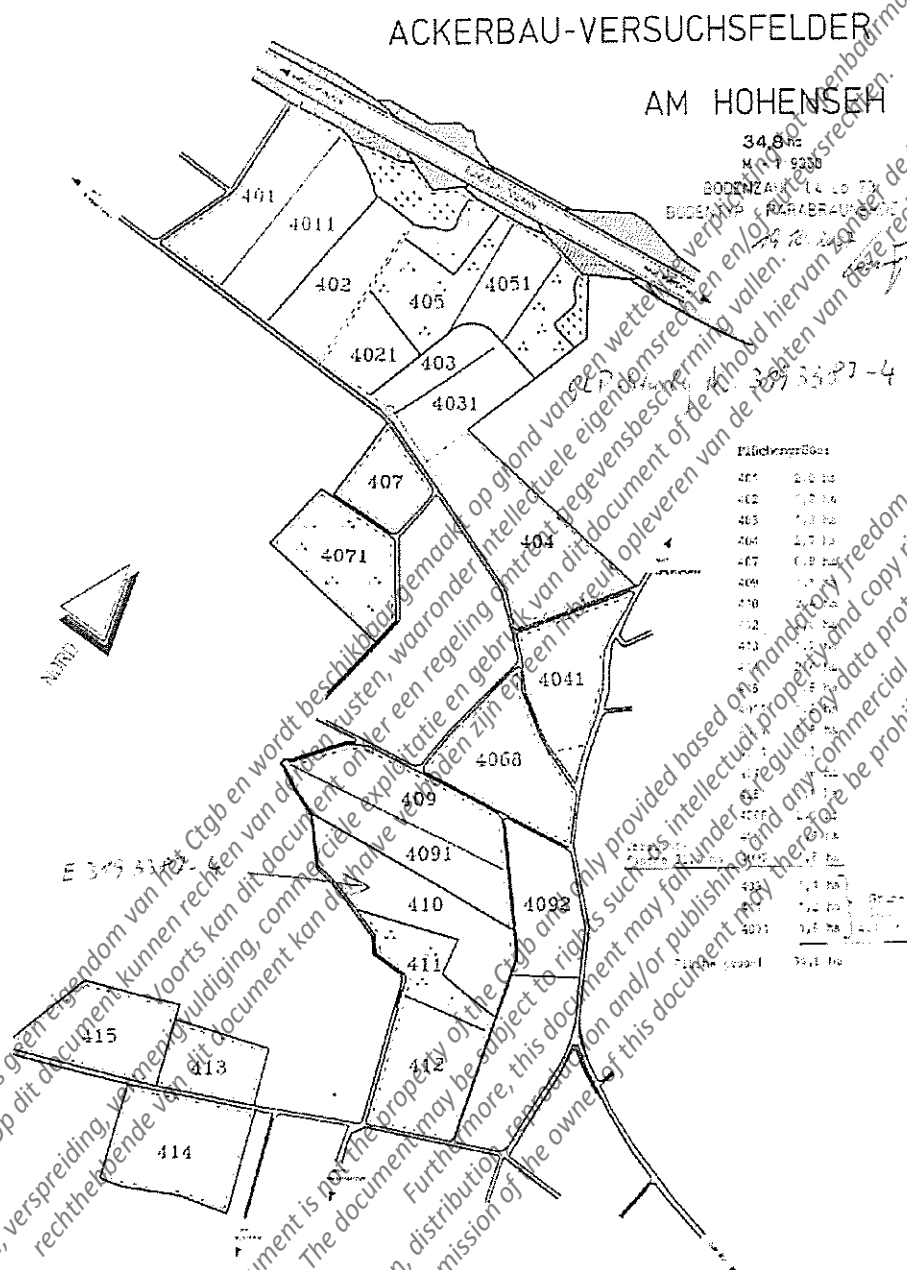
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13 APPENDICES

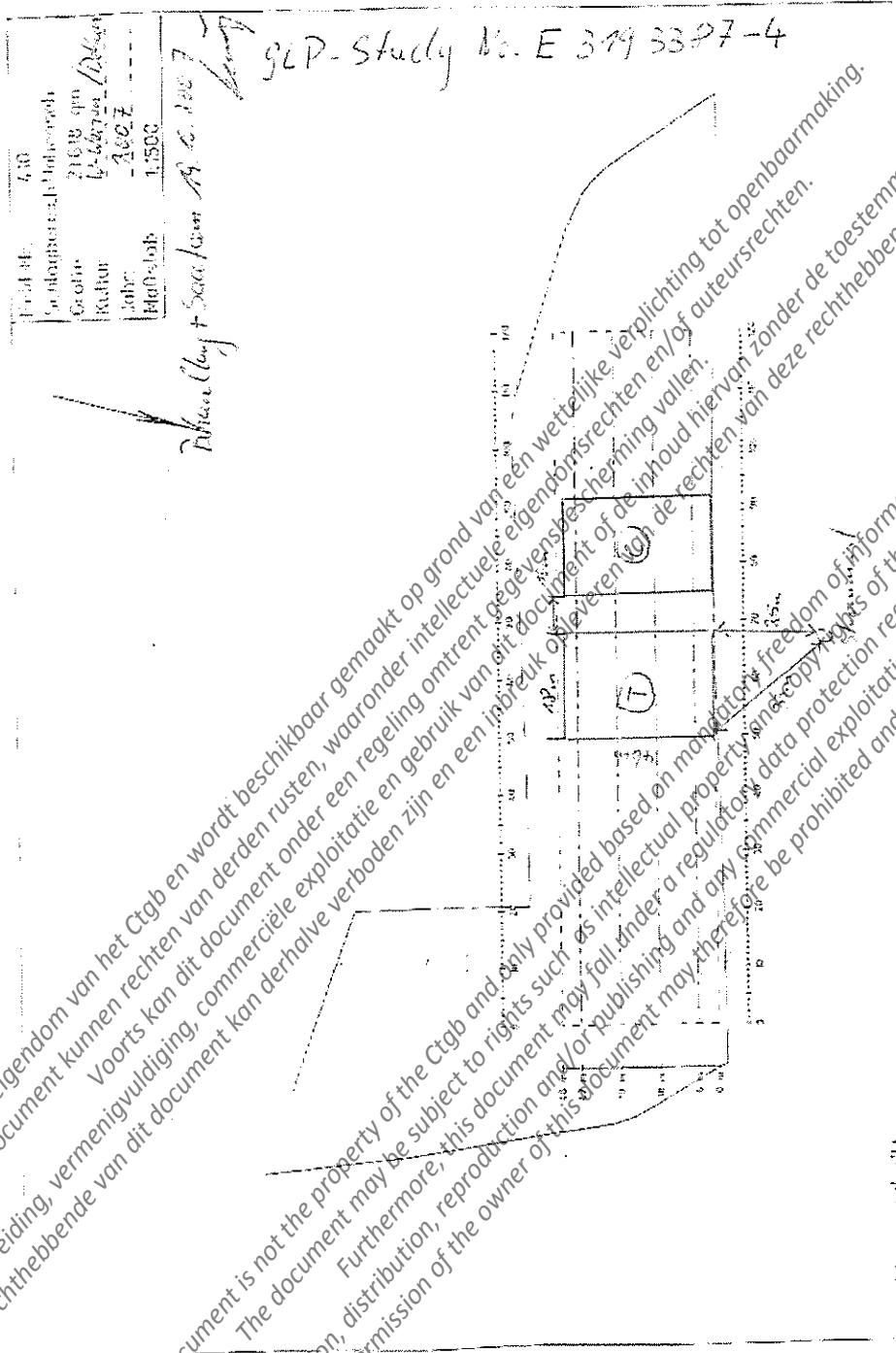
Appendix 1 Study Plot Location

1



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Appendix 1 Study Plot Location (continued)



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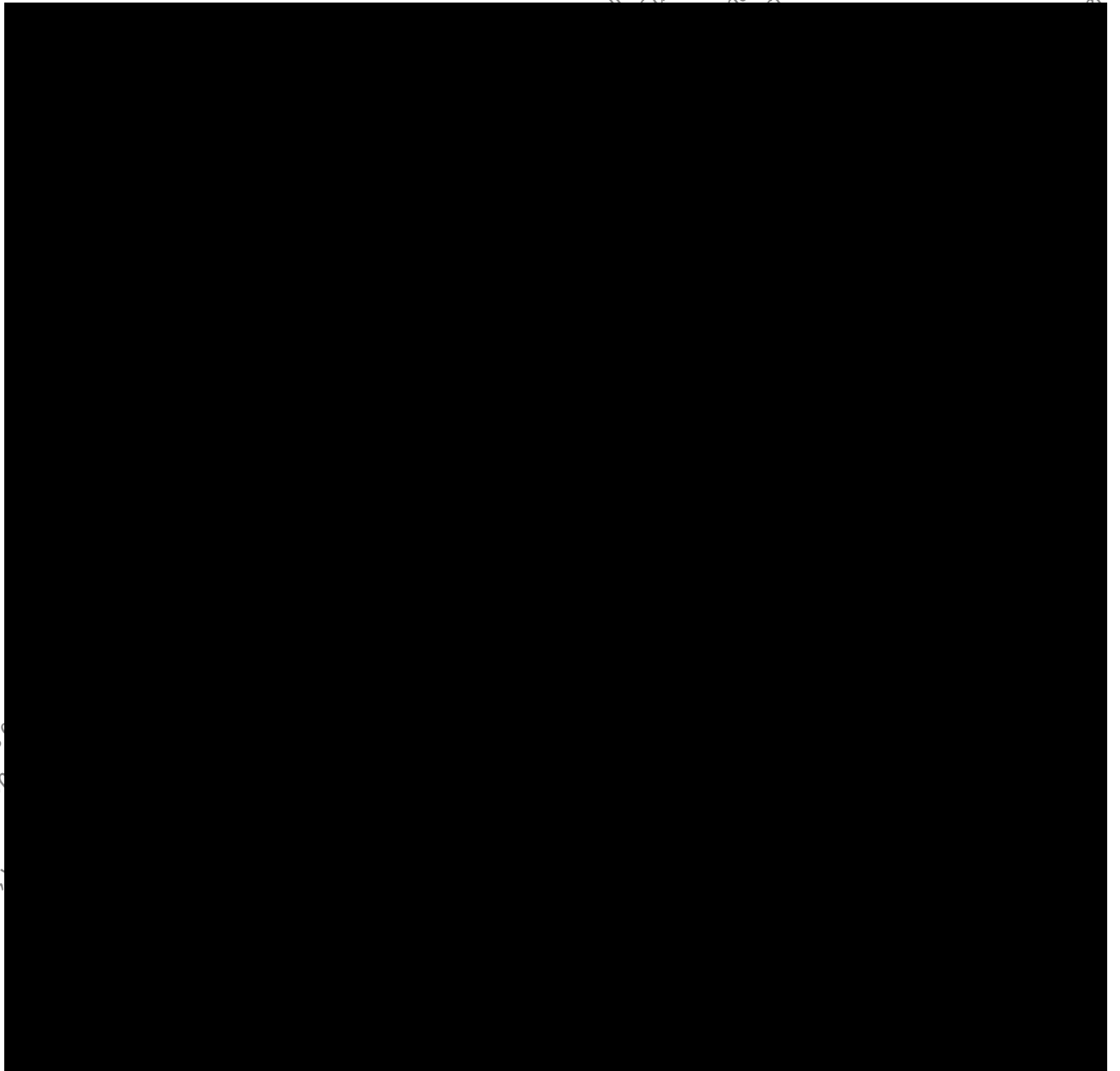
Appendix 2 Certificate of Analysis of the Test Item

BCS-D-FT Analysis&Services
Hoppe, Monika : Alfred-Nobel-Strasse 50
GEB.6820 ; 40789 Monheim
Phone : +49 2173 / 38-2292



Bayer CropScience

Certificate of Analysis

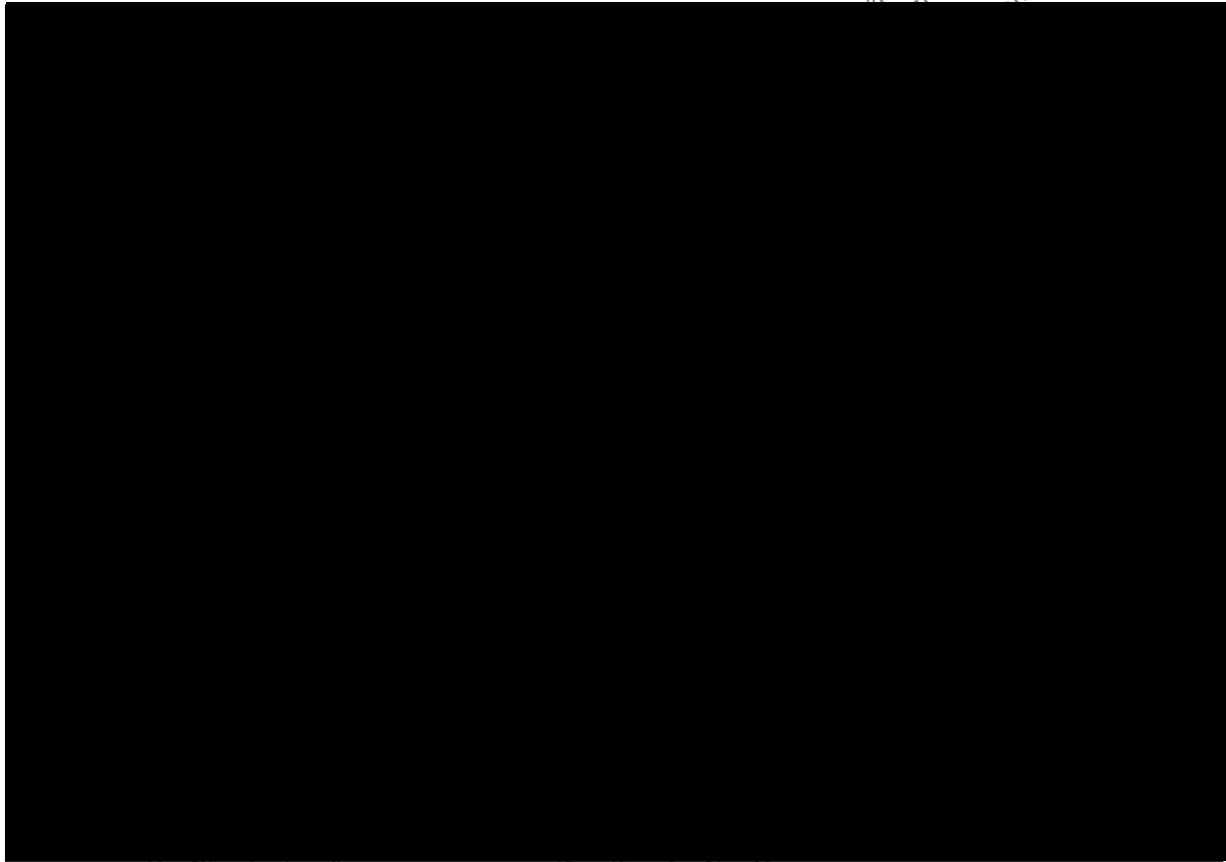


Appendix 2 Certificate of Analysis of the Test Item (continued)

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Batch-ID : 2007-009957
Sample description : TOX 08068-00

Page 2

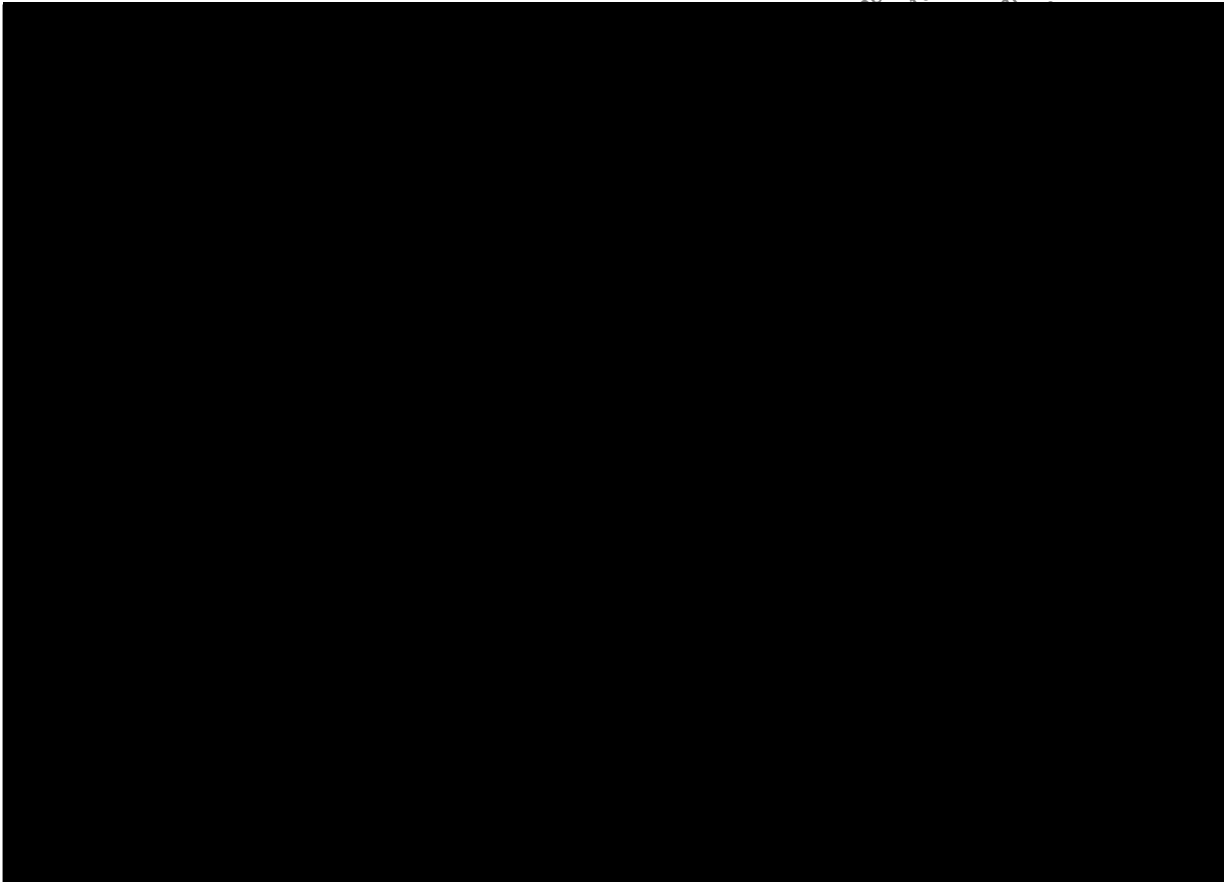
end of report

Appendix 3 Certificate of Analysis of Dressed Winter Wheat Seeds

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Sample description: FOX08079-00

Page: 1

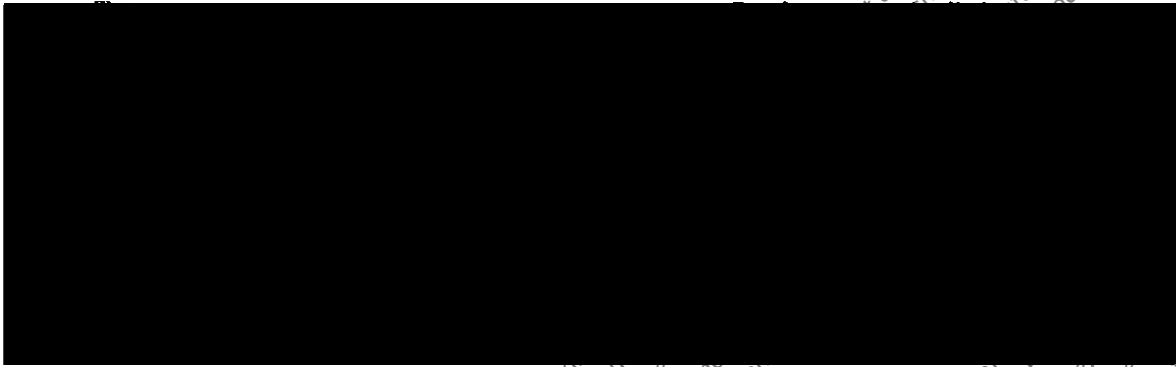
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Appendix 3 Certificate of Analysis of Dressed Winter Wheat Seeds (continued)

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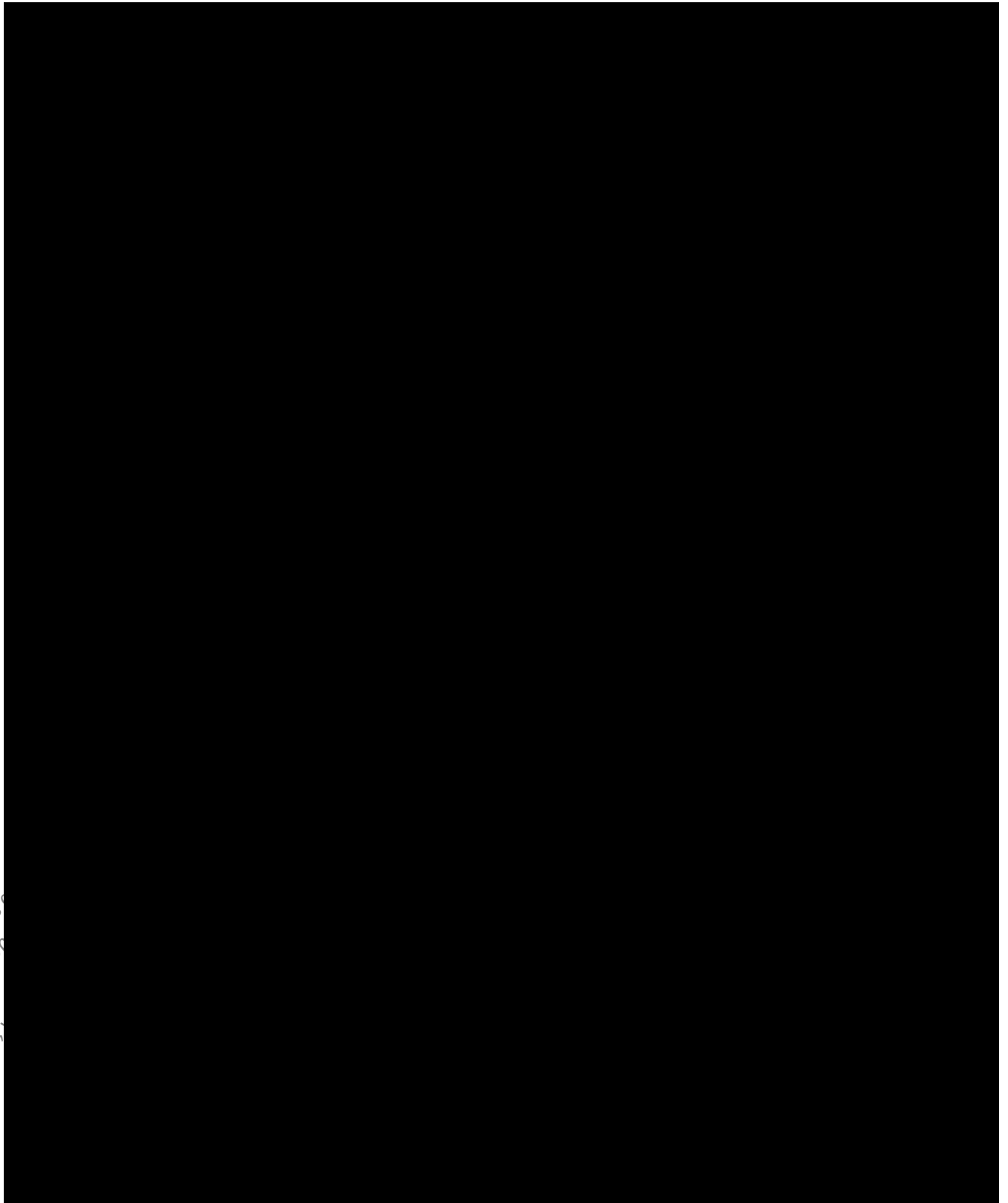


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Sample description : TOX08079-00

Page: 2

end of report

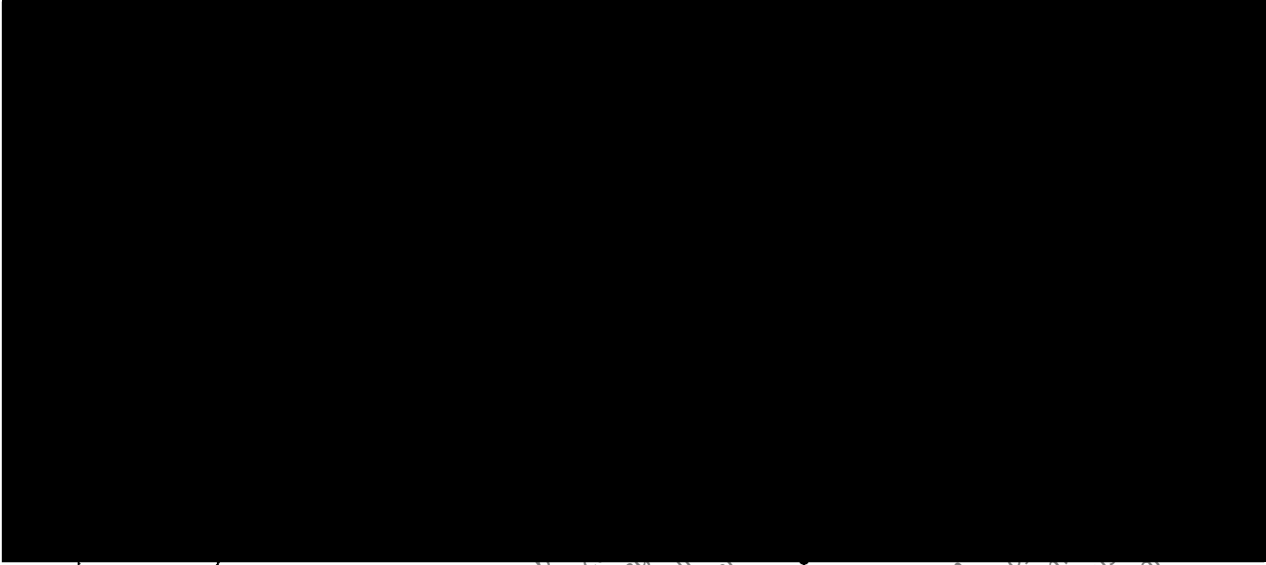
Appendix 4 GLP Certificate BCS-D-EnSa-ETX



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Appendix 5 Analytical Phase Report Soil Analysis

Bayer CropScience AG
BCS-D-HS-RA
D-40789 Monheim am Rhein

Lynx-ID: EBNTL006
Study No.: E 319 3387-4

Analytical Report

Study Title

Determination of residue levels of imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine in bee relevant matrices of winter rape in a cereal succeeding crop scenario at Bayer CropScience AG experimental farm "Höfchen", Germany

Purpose

According to EU directive 91/414/EEC the possible adverse effects of pesticides on wildlife have to be examined. This study aims to determine the exposure of honeybees to imidacloprid and its metabolites imidacloprid-monohydroxy and imidacloprid-olefine by potential uptake of soil residues of imidacloprid into bee-relevant matrices of winter rape plants in a succeeding crop scenario.

Data Requirement

EU-Ref: Council Directive 91/414/EEC of July 15, 1991

Author

[REDACTED]

Report Completion Date

2011-01-13

Date: yyyy-mm-dd

Analytical Test Site

Bayer CropScience AG
Development – Human Safety – Residue Analysis
(formerly Development – Residues, Operator and Consumer Safety)
BCS-D-HS-RA
Alfred-Nobel-Str. 50
D-40789 Monheim am Rhein

Sponsor

Bayer CropScience AG
Alfred-Nobel-Str. 50
D-40789 Monheim am Rhein



M-400720-01-1

Appendix 5 Analytical Phase Report Soil Analysis (continued)**Bayer CropScience AG
BCS-D-HS-RA****Study No.: E 319 3387-4**

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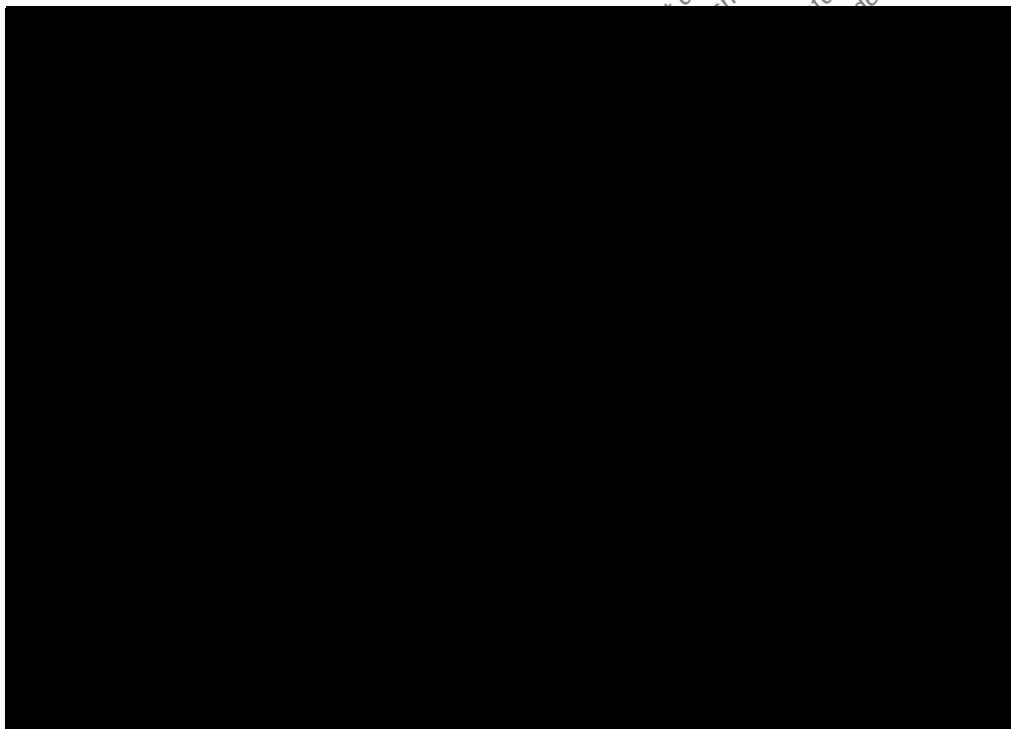
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Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Certification of Good Laboratory Practice



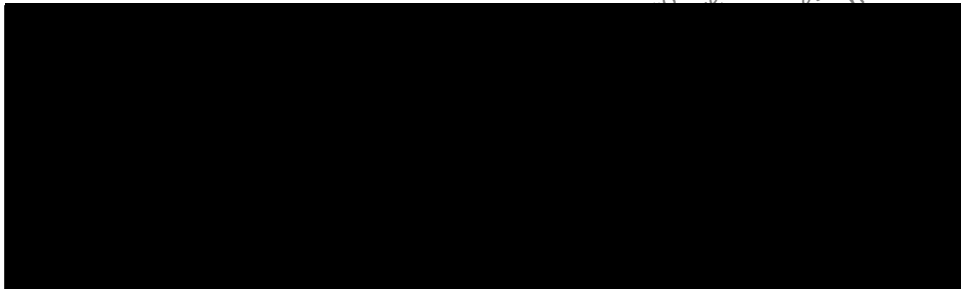
If not otherwise stated, in this report the **date format yyyy-mm-dd** is used.

Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Certification of Authenticity



Inquiries should be directed to:



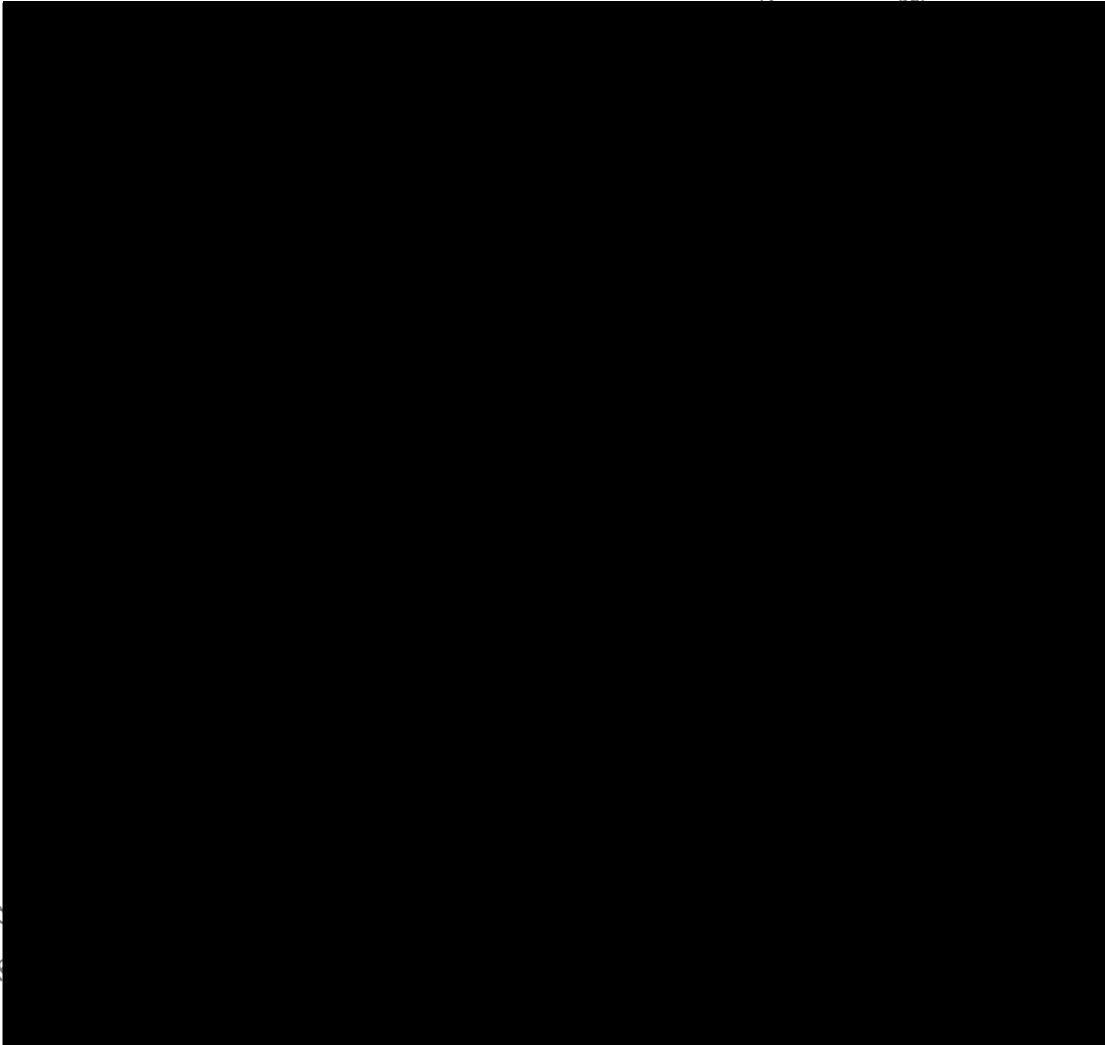
Bayer CropScience AG
Alfred-Nobel-Str. 50
BCS-D-HS-RA
Building 6610
D-40789 Monheim am Rhein



Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4



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Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

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Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

1 Summary

The purpose of the study was to examine the exposure of honeybees to imidacloprid and its metabolites imidacloprid-hydroxy and imidacloprid-olefine by potential uptake of soil residues of imidacloprid into bee-relevant matrices of winter rape plants in a succeeding crop scenario. This analytical report describes the results of the determination of residues of imidacloprid in soil.

The study was conducted on the field area of the Bayer CropScience experimental farm Höfchen, close to Burscheid (Nordrhein-Westfalen, Germany). One plot was treated with Manta Plus FS 145.2 G (Fuberidazole + Imazalil + Imidacloprid + Triadimenol FS 145.2 (7.2+8+70+60) G) (spray application and incorporation into soil) and sown with Manta Plus FS 145.2 G dressed winter-wheat seeds. To determine the actual imidacloprid concentration in soil after application and incorporation into 20 cm depth, 15 soil samples from spots distributed all over the treated plot were taken by a hydraulic sampling device. Due to technical reasons only soil samples of 30 cm depth could be taken by this method, so that the lower 10 cm of each soil sample were discarded, the upper 20 cm of each sample were pooled and mixed for one single soil sample for analysis. All samples were dispatched to the Laboratory for Sampling, Preparation Technique and Sample Logistics (PVT/L), Bayer CropScience AG in D-40789 Monheim am Rhein where they were stored until analysis at -18°C or below in dark conditions.

Residues of imidacloprid in/on soil were determined according to method 00790/M001.

Soil samples of 25 g were extracted with acetonitrile / water (3 / 7 parts by volume) for 3 minutes in a microwave at 250W. After the extraction internal standard was added for quantification to compensate possible matrix effects in the MS-detector. The extract was shaken 1 minute for homogenization. An aliquot of 1.5 mL was taken and centrifuged. The supernatant was taken and injected into the HPLC-MS/MS system. Two replicates (A + B) of 25 g each were analysed from each sample. The mean value of both replicates is reported.

The limit of quantitation (LOQ) of the method was 5.0 µg/kg for imidacloprid corresponding to the lowest fortification level of successfully conducted recovery experiments.

For Soil the individual recovery values for imidacloprid ranged from 82 to 94% with an overall recovery of 89% and with a relative standard deviation (RSD) of 7.0% (n = 4).

All results of the method validation were in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully.

The analytical results for imidacloprid in soil are reported in Table 3 under section 6.3.

Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

2 Organisation and Staff

2.1 Organisation of Project

Sponsor Bayer CropScience AG
Alfred-Nobel-Str. 50
D-40789 Monheim am Rhein

Study Director [REDACTED]
Bayer CropScience AG
BCS-D-ENSA-ETX (since 2009, Feb 1)
Alfred-Nobel-Str. 50
D-40789 Monheim am Rhein

Analytical Test Site Bayer CropScience AG
BCS-D-HS-RA (since 2009, Feb 1)
Alfred-Nobel-Str. 50
D-40789 Monheim am Rhein

Test Facility BCS-D-ENSA-ETX (since 2009, Feb 1)
Alfred-Nobel-Str. 50
D-40789 Monheim am Rhein

2.2 Responsible Personnel for Residue Analysis

Head of Analytical Test Site [REDACTED]
Bayer CropScience AG
BCS-D-HS-RA
Alfred-Nobel-Str. 50
D-40789 Monheim am Rhein

PI Analysis and Head of Laboratory [REDACTED]
Bayer CropScience AG
BCS-D-HS-RA
D-40789 Monheim am Rhein

Technician [REDACTED]
Bayer CropScience AG
BCS-D-HS-RA
D-40789 Monheim am Rhein

2.3 Responsible Personnel for the Field Part

Representative Sponsor of the Field Part Bayer CropScience AG
BCS-D-EnSa-ETX
Alfred-Nobel-Str. 50
D-40789 Monheim am Rhein

Appendix 5 Study Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

2.4 Archiving

All raw data pertaining to the analytical phase of this study and the final report of the analytical phase are stored in the central GLP archive of Bayer CropScience AG, Alfred-Nobel-Str. 50, D-40789 Monheim am Rhein for as long as required by GLP principles.

Reserve samples of the reference items are stored in the archives of Bayer CropScience AG, Product Technology-Analytics Frankfurt, Industriepark Höchst, D-65926 Frankfurt, and of Bayer CropScience AG, Research-Product Technology, Isotope Chemistry, Aprather Weg 18a, D-42096 Wuppertal. The test and reference items are stored as long as their quality still guarantees an evaluation.

2.5 Quality Assurance Unit

Bayer CropScience AG
BCS-D-SPQ-GLP/QA
Agricultural Centre Monheim
Alfred-Nobel-Str. 50
D-40789 Monheim am Rhein

2.6 Schedule

Approval of Study by the Study Director	2007-09-18
Start of Analytical Phase (First extraction)	2009-02-10
End of Analytical Phase (Last printout of a chromatogram)	2009-03-13

3 Study Objective

The purpose of the study was to examine the exposure of honeybees to imidacloprid and its metabolites imidacloprid-hydroxy and imidacloprid-olefine by potential uptake of soil residues of imidacloprid into bee-relevant matrices of winter rape plants in a succeeding crop scenario. This analytical report describes the results of the determination of residues of imidacloprid in soil.

Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

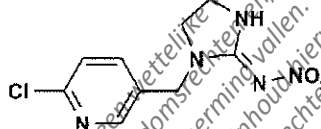
Study No.: E 319 3387-4

4 Compounds

4.1 Reference Items

Name of the Substance	Imidacloprid
Chemical Name	1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine
Empirical Formula	C ₉ H ₁₀ ClN ₅ O ₂
Mol ID	84

Structural Formula

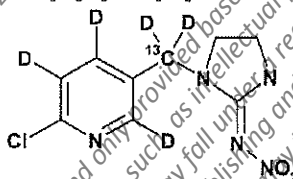


Molar Mass	255.69 g/mol
Analytical Certificate No.	AZ 13319, dated 2006-05-08
Batch No.	EDE0002474
Purity	98.7%
Expiration Date	April 2009
Storage Conditions	+5°C ± 5°C

4.2 Internal Standards

Name of the Substance	Imidacloprid-d₅¹³C
Chemical Name	[pyridine-d ₃ , pyridinylmethyl-d ₂ , ¹³ C]
Empirical Formula	¹³ C ₉ H ₅ d ₅ ClN ₅ O ₂

Structural Formula



Molar Mass	258.3 g/mol
Analytical Certificate No.	BECH 1272-1-1 from 2008-09-02
Batch No.	BECH 1272-1-1
Storage Conditions	-20°C ± 5°C

Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

5 Sample Material and Sample Preparation

The study was conducted on the field area of the Bayer CropScience experimental farm Höfchen, close to Burscheid (Nordrhein-Westfalen, Germany). One plot was treated with Manta Plus FS 145.2 G (Fuberidazole + Imazalil + Imidacloprid + Triadimenol FS 145.2 (7.2+8+70+60) G) (spray application and incorporation into soil) and sown with Manta Plus FS 145.2 G dressed winter-wheat seeds. To determine the actual imidacloprid concentration in soil after application and incorporation into 20 cm depth, 15 soil samples from spots distributed all over the treated plot were taken by a hydraulic sampling device. Due to technical reasons only soil samples of 30 cm depth could be taken by this method, so that the lower 10 cm of each soil sample were discarded, the upper 20 cm of each sample were pooled and mixed for one single soil sample for analysis. All samples were dispatched to the Laboratory for Sampling, Preparation Technique and Sample Logistics (PVTL), Bayer CropScience AG in D-40789 Monheim am Rhein where they were stored until analysis at -18°C or below in dark conditions.

An overview of the samples generated is shown in Table 1.

Table 1: Summary of Field Samples Soil

Sample Code	Sample Material Name	Sample Material Type	Treated/Control Plot [T/C]	Sample Weight [kg]
E-3193387-4 T01	Soil	0 – 20 cm	T	9.9
E-3193387-4 T02		0 – 20 cm	T	9.25
E-3193387-4 T02K		0 – 20 cm	C	9.45

Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

6 Residue Analyses

6.1 Analytical Method

Method No. 00790/M001
Author [REDACTED]
Citation MR-106/03, dated on 2004-04-02

Residues of imidacloprid in/on soil were determined according to method 00790/M001.

Soil samples of 25 g were extracted with acetonitrile / water (3 / 7 parts by volume) for 3 minutes in a microwave at 250W. After the extraction internal standard was added for quantification to compensate possible matrix effects in the MS-detector. The extract was shaken 1 minute for homogenization. An aliquot of 1.5 mL was taken and centrifuged. The supernatant was taken and injected into the HPLC-MS/MS system. Two replicates (A + B) of 25 g each were analysed from each sample. The mean value of both replicates is reported.

Detailed information of the HPLC and MS/MS conditions are given in Appendix 2.

The limit of quantitation (LOQ) of the method was 5.0 µg/kg for imidacloprid corresponding to the lowest fortification level of successfully conducted recovery experiments. Representative linearity plots are given in

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Appendix 5 Analytical Phase Report Soil Analysis (continued)**Bayer CropScience AG
BCS-D-HS-RA****Study No.: E 319 3387-4**

Appendix 3.

Data on method validation are given in Chapter 6.2.

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Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

6.2 Method Validation

The analytical method was validated by running concurrent recoveries at the LOQ and higher residues levels. Therefore control samples were spiked with imidacloprid. Fortification levels and recovery data are given in Table 2.

Table 2: Recovery Data for Imidacloprid in/on Soil,
FL: Fortification Level, RSD: Relative Standard Deviation, LOQ: Practical Limit of Quantitation.

Sample Material	Fortification Level [µg/kg]	Recoveries - Single Values [%]	Mean [%]	RSD [%]
Control soil Höfchen (Plot 4011)	5	82	-	-
	50	94	-	-
	Mean and RSD [%]		88	-
Control soil Laacherhof	5	85	-	-
	50	94	-	-
	Mean and RSD [%]		90	-
	Overall Mean and RSD [%]		89	7.0

Note: Fortified, determined and calculated as imidacloprid.

For Soil the individual recovery values for imidacloprid ranged from 82 to 94% with an overall recovery of 89% and with a relative standard deviation (RSD) of 7.0% (n = 4).

All results of the method validation were in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully.

Representative chromatograms are included in Appendix 1.

6.3 Results

Table 3 shows the results obtained for the nectar and pollen samples.

Table 3: Analytical Results for Imidacloprid in Soil

Sample Code	T / C	Date of Extraction	Actual concentration of Imidacloprid					
			Wet soil [µg/kg]			Dry soil [µg/kg]		
			Sample 1	Sample 2	Mean	Sample 1	Sample 2	Mean
E-3193387-4 T01	T	2009-03-13	27.0	27.6	27.3	33.6	34.4	34.0
E-3193387-4 T02	T	2009-03-13	12.2	12.1	12.1	15.3	15.1	15.2
E-3193387-4 T02K	C	2009-03-13	0	0	0	<LOD	<LOD	<LOD

Remark: T = Treated C = Control

The limit of quantitation (LOQ) of the method is 5 µg/kg for Imidacloprid.

The limit of detection (LOD) of the method is 2 µg/kg for Imidacloprid.

Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

7 Evaluation and Discussion

For Soil the individual recovery values for imidacloprid ranged from 82 to 94% with an overall recovery of 89% and with a relative standard deviation (RSD) of 7.0% (n = 4).

All results of the method validation were in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully.

The analytical results for imidacloprid in soil are reported in Table 3 under section 6.3.

8 References

- [1] Analytical Method 00790/M001, (MR-106/03) for the Determination of Residues of Imidacloprid in Soil by HPLC-MS/MS, [redacted] unpublished report of Bayer CropScience AG, Report No. MR-106/03, 2004-04-02.

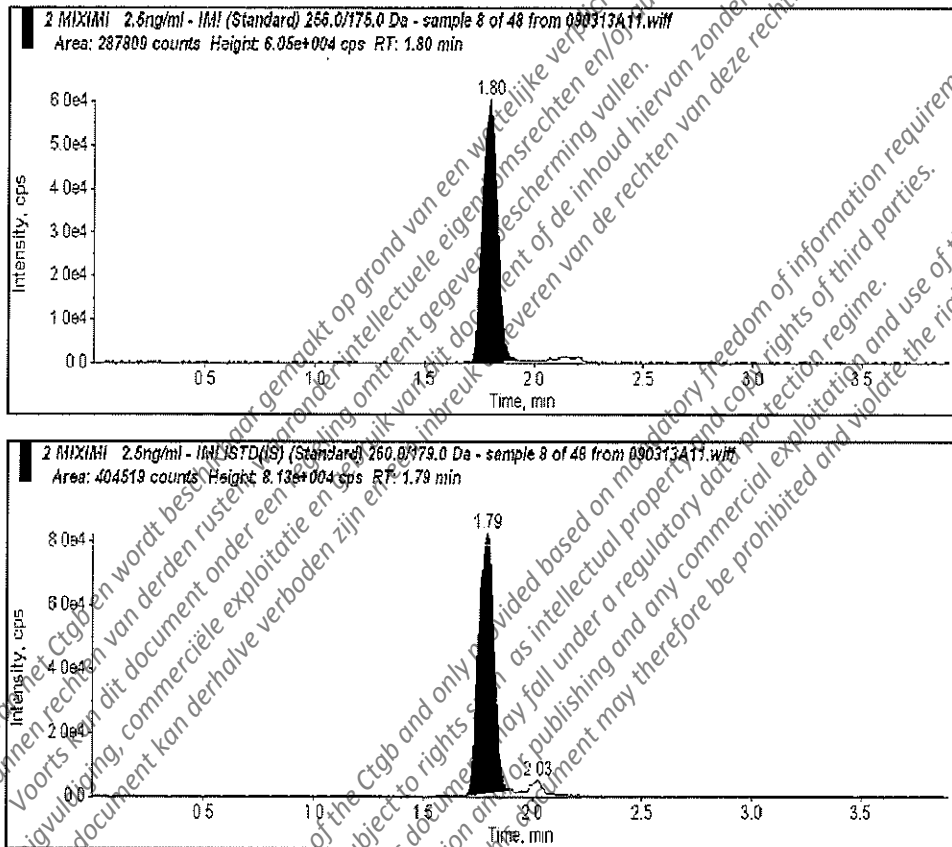
Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms

Figure 1 : Standard Solution Imidacloprid 2.5 µg/L and Imidacloprid-d₅¹³C 2.5 µg/L in Solvent
Top: Imidacloprid,
Bottom: Internal Standard Imidacloprid-d₅¹³C



Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

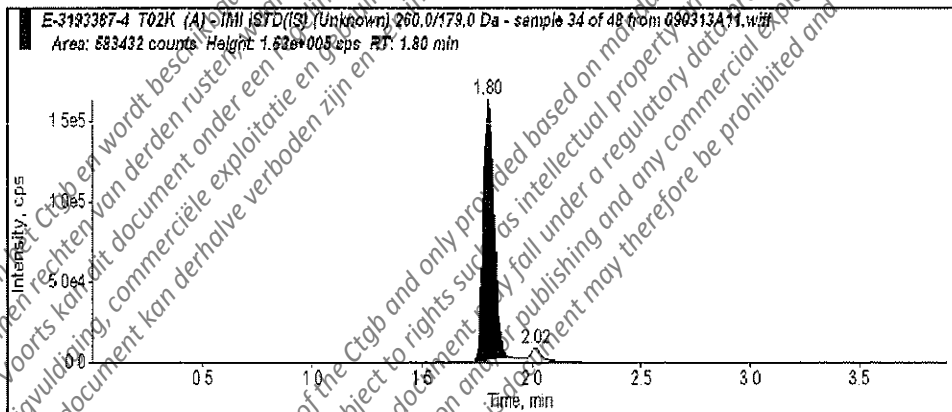
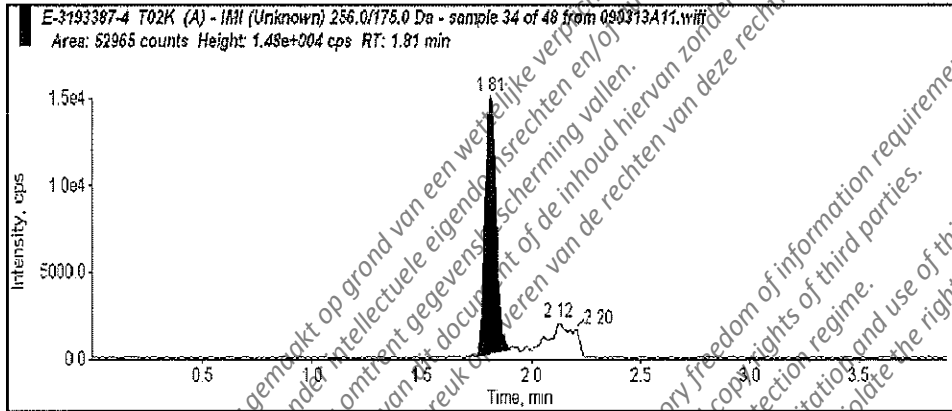
Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (contd)

Figure 2 : Control Sample Soil, E-3193387-4 T02K

Top: Imidacloprid

Bottom: Internal Standard Imidacloprid-d₅¹³C



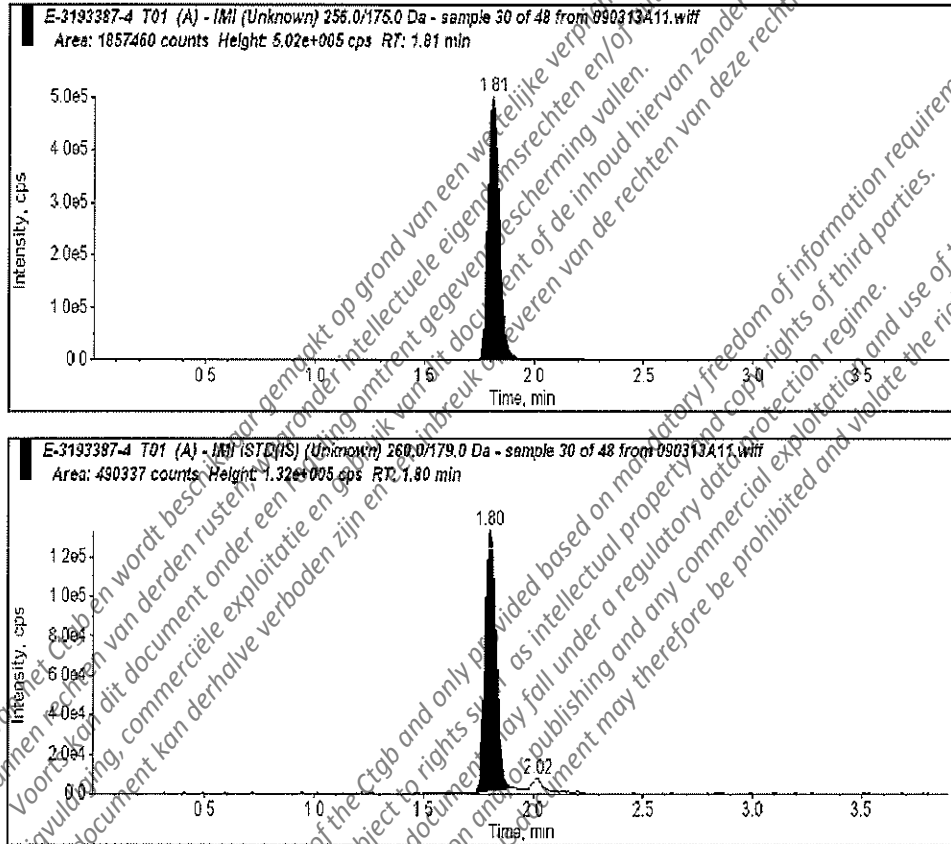
Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (contd)

Figure 3 : Treated Sample Soil, E-3193387-4 T01
Top: Imidacloprid,
Bottom: Internal Standard Imidacloprid-d₅¹³C



Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

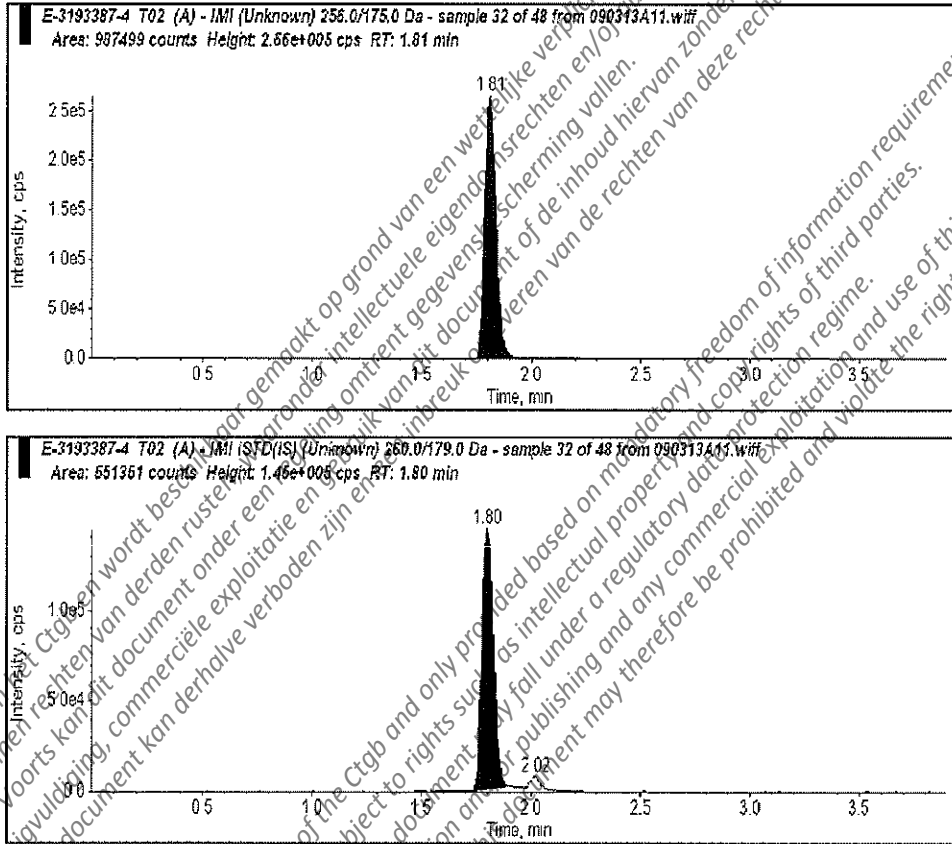
Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (cont'd)

Figure 4 : Treated Sample Soil, E-3193387-4 T02

Top: Imidacloprid,

Bottom: Internal Standard Imidacloprid- d_5 ^{13}C



Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 2: Detailed Instrument Parameters

2009-03-13 [Redacted]

Study: E 319 3387-4

Technician: M. Schnöder

Printing Time: 2:35:42 PM

Acquisition Method
2009-12-04 IPI + ISID - Q RM
Mass Spec 3.991 min
Period 3.501 min
+RM
Agilent 1200 Binary Pump (4.0 mins)
Equilibrate (0.0 mins)
Run (4.0 mins)
Agilent 1200 Isocratic Pump (4.0 mins)
Equilibrate (0.0 mins)
Run (4.0 mins)
Agilent 1200 Thermostated Column Compartment
Integrated Valve
CIV PAL Autosampler

Acquisition Method Properties
Column: Luna HSD (Standard)
Injection Mode: In Sync
Auto-Equilibration: Off
Acquisition Direction: Autoset
Number of Scans: 713
Periods in File: 1
Acquisition Method: Acquisition Method
Software Version: Analyst 1.5

*Solvent B1 (Bin Pump): MQ-Water/ACN 1/9 +2ml formic acid/L
*Solvent A1 (Bin Pump): MQ-Water/ACN 9/1 +2ml formic acid/L
*Solvent Isocratic Pump: MQ-Water/ACN 9/2 +0.1ml acetic acid/L

API 4000 Serial Number: V08780410

Page 1 of 5

Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 2: Detailed Instrument Parameters (contd)

2809-03-13

Study: E 319 3387-4

Technician: U. Schneider

Printing Time: 2:35:42 PM

Acquisition Method

2809-13-04.MU + TSD1 + Q.PRM

Mass Spec 3.901 min
RetTime 3.901 min
#000

Agilent: 1200 Binary Pump (4.0 min)
Equilibrate (0.0 min)
Run (4.0 min)

Agilent: 1200 Fraction Pump (4.0 min)
Equilibrate (0.0 min)
Run (4.0 min)

Agilent: 1200 Thermoatted Column Cartridge
Integrated Valve Valve
GC MS Autosampler

- * Solvent B1 (Bin Pump): MQ-Water/ACN 1/9 +2ml formic acid/L
- * Solvent A1 (Bin Pump): MQ-Water/ACN 9/1 +2ml formic acid/L
- * Solvent Isocratic Pump: MQ-Water/ACN 9/2 +0.1ml acetic acid/L

API 4000 Serial Number: VB6750410

Page 2 of 5

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Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 2: Detailed Instrument Parameters (contd)

2009-03-13

Study: E 319 3387-4

Technician: U.Schneider

Printing Time: 2:35:42 PM

Acquisition Information:

Acquisition Method: 2008-12-04 DME + ISID + Q ES24.Dm
 Created: Friday December 05 2008 06: 11: 39 AM
 Last Modified: Tuesday December 09 2008 17: 08: 56 EM
 Comment:
 Synchronization Mode: IC Sync
 Auto-Equilibration: OFF
 Acquisition Duration: 4min00sec
 Number Of Scans: 743
 Periods In File: 1
 Acquisition Module: Acquisition Module
 Software Version: Analyst 1.0

Period 1:

Scans In Period: 743
 Relative Start Time: 0.000000
 Experiments In Period: 1

Period 1 Experiment 1:

Scan Type: 004 0000
 Scheduled M/N: No
 Polarity: Positive
 Scan Mode: 000
 Ion Source: Auto Spray
 Resolution Q1: Unit
 Resolution Q3: Unit
 Intensity Thres.: 0.00 cps
 Settling Time: 0.0000 msec
 MR Mass: 5.0070 msec
 MCA: No
 Step Size: 0.00 Da

Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	Range	Start	Stop	IL
256.00	175.00	100.00	CE	21.00	27.00	10%
			CF	16.00	16.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	Range	Start	Stop	IL
256.00	269.00	100.00	CE	23.00	23.00	10%
			CF	14.00	14.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	Range	Start	Stop	IL
260.00	175.00	100.00	CE	27.00	27.00	10% ISID
			CF	16.00	16.00	

Parameter Table (Period 1 Experiment 1):

SAP: 6.00
 CUP: 35.00
 GSI: 50.00
 GSI2: 50.00
 IS: 5500.00
 TSP: 500.00
 SVA: 01
 RF: 51.00
 RF: 10.00

Agilent LC Trap Method Properties

*Solvent B1 (Bin Pump): MQ-Water/ACN 1/9 +2ml formic acid/L
 *Solvent A1 (Bin Pump): MQ-Water/ACN 9/1 +2ml formic acid/L
 *Solvent Isocratic Pump: MQ-Water/ACN 3/2 +0.1ml acetic acid/L
 API 4000 Serial Number V08750410
 Page 3 of 5

Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 2:
Detailed Instrument Parameters (contd)

2008-03-13

Study: E 319 3387-4

Technician: U.Schneider

Printing Time: 2:35:42 PM

Agilent 1200 Binary Pump

Minimum Pressure (psl): 0.0
 Maximum Pressure (psl): 5801.0
 Dead Volume (ul): 40.0
 Maximum Flow Pump (ml/min): 100.0
 Maximum Pressure Pump (psl/sec): 290.0

Flow Table:

Step	Total Time (min)	Flow Rate (ul/min)	A (%)	B (%)
0	0.00	500	95.0	5.0
1	0.20	500	95.0	5.0
2	2.20	500	5.0	95.0
3	2.90	500	5.0	95.0
4	3.00	500	95.0	5.0
5	4.00	500	95.0	5.0

Left Compressibility: 50.0
 Right Compressibility: 115.0
 Left Dead Volume (ul): 40.0
 Right Dead Volume (ul): 40.0
 Left Stroke Volume (ul): -1.0
 Right Stroke Volume (ul): -0.0
 Left Solvent: B1
 Right Solvent: B1

Agilent 10 Pump Method Properties

Pump Model: Agilent 1200 Isocratic Pump
 Minimum Pressure (psl): 0.0
 Maximum Pressure (psl): 5801.0
 Compressibility: 100.0
 Dead Volume (ul): 40.0
 Stroke Volume (ul): -1.0
 Maximum Flow Pump (ml/min): 100.0
 Maximum Pressure Pump (psl/sec): 290.0

Flow Table:

Step	Total Time (min)	Flow Rate (ul/min)	IN1	IN2	IN3	IN4
0	0.00	500	open	open	open	open
1	0.20	500	open	open	open	open

Agilent Column Oven Properties

Left Temperature (°C): 60.00
 Right Temperature (°C): 60.00
 Temperature Tolerance +/- (°C): 1.00
 Sample Acquisition Tolerance +/- (°C): 0.50
 Valve: None Used
 Column Selection (valve): Installed
 Position for first sample in the batch: Left
 Use same position for all samples in the batch.

Valve Valve

Step	Total Time (min)	Position
1	1.3	PS
2	2.2	None

CIC 101 Autosampler Method Properties
 Loop Volume (ul): 100
 Loop Volume 2 (ul): 100

* Solvent B1 (Bin Pump): MQ Water/ACH 1/9 +2ml formic acid/L
 * Solvent A1 (Bin Pump): MQ Water/ACH B1 +2ml formic acid/L
 * Solvent Isocratic Pump: MQ Water/ACH B2 +0.1ml acetic acid/L

API 4000 Serial Number V08780410

Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 2: Detailed Instrument Parameters (contd)

2009-03-13

Study: E 319 3387-4

Technician: U. Schuelder

Printing Time: 2:35:42 PM

```

Injection Volume (µL): 30.001
Method Description:

Syringe: 100µL
01 Analyst: IC-Intj
Air Volume (µL)
Pre-Clean with Solvent 1 ( )
Pre-Clean with Solvent 2 ( )
Pre-Clean with Sample ( )
Filling Speed (µL/s)
Filling Strokes ( )
Inject to
Injection Speed (µL/s)
Pre Inject Delay (ms)
Post Inject Delay (ms)
Post Clean with Solvent 1 ( )
Post Clean with Solvent 2 ( )
Valve Clean with Solvent 1 ( )
Valve Clean with Solvent 2 ( )
Replicate Count ( )
Analysis Time (s) ( )

```

*Solvent B1 (Bin Pump): MQ-Water/ACN 1/9 +2ml formic acid/L
 *Solvent A1 (Bin Pump): MQ-Water/ACN 8/1 +2ml formic acid/L
 *Solvent Isocratic Pump: MQ-Water/ACN 8/2 +0.1ml acetic acid/L

API 4000 Serial Number: VBS750410

Page 6 of 8

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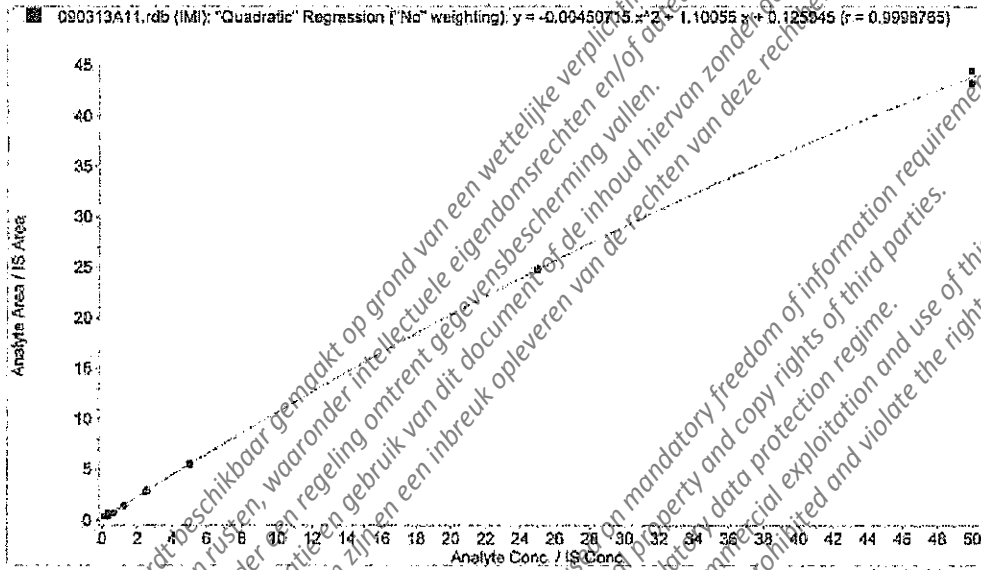
Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 3: Representative Linearity Plots

Figure 5 : Linearity Imidacloprid (Range of Concentration: 1.25 - 200 ng/mL)



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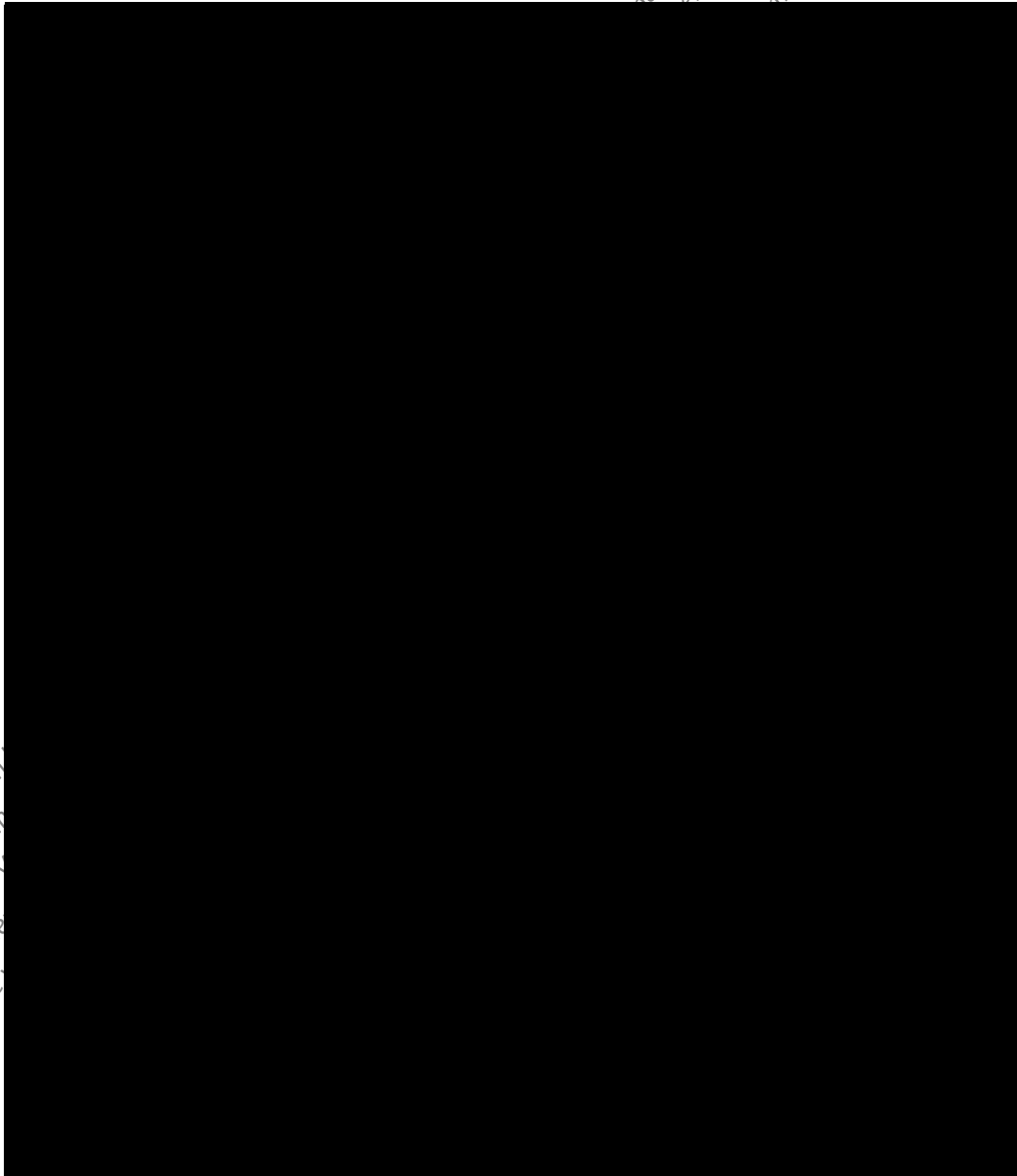
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Appendix 5 Analytical Phase Report Soil Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 4: GLP Certificate



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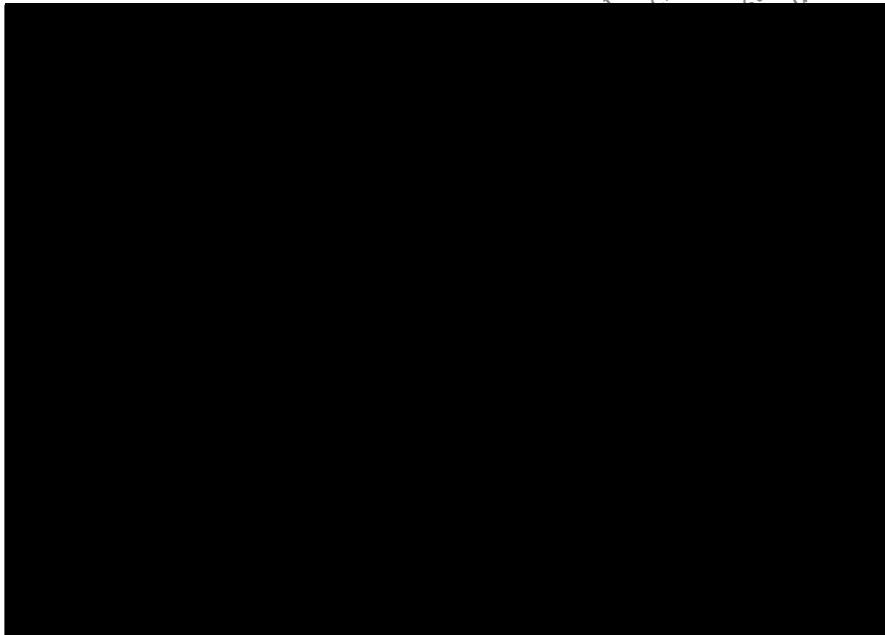
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Appendix 5 Analytical Phase Report Soil Analysis (continued)

**Bayer CropScience AG
BCS-D-HS-RA**

Study No.: E 319 3387-4

**Appendix 4:
GLP Certificate (contd)**



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Appendix 6 Analytical Phase Report Nectar and Pollen Analysis

Bayer CropScience AG
BCS-D-HS-RA
D-40789 Monheim am Rhein

Lynx-ID: EBNTL006
Study No.: E 319 3387-4

Analytical Report

Study Title

Determination of residue levels of imidacloprid, imidacloprid-monohydroxy and imidacloprid-olefine in bee relevant matrices of winter rape in a cereal succeeding crop scenario at Bayer CropScience AG experimental farm "Höfchen", Germany

Purpose

According to EU directive 91/414/EEC the possible adverse effects of pesticides on wildlife have to be examined. This study aims to determine the exposure of honeybees to imidacloprid and its metabolites imidacloprid-monohydroxy and imidacloprid-olefine by potential uptake of soil residues of imidacloprid into bee-relevant matrices of winter rape plants in a succeeding crop scenario.

Data Requirement

EU-Ref. Council Directive 91/414/EEC of July 15, 1991

Authors



Report Completion Date

2010-01-11
Date: yyyy-mm-dd

Analytical Test Site

Bayer CropScience AG
Development – Human Safety – Residue Analysis
(formerly Development – Residues, Operator and Consumer Safety)
BCS-D-HS-RA
Alfred-Nobel-Str. 50
D-40789 Monheim am Rhein

Sponsor

Bayer CropScience AG
Alfred-Nobel-Str. 50
D-40789 Monheim am Rhein

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)**Bayer CropScience AG
BCS-D-HS-RA****Study No.: E 319 3387-4**

Data Confidentiality Statement

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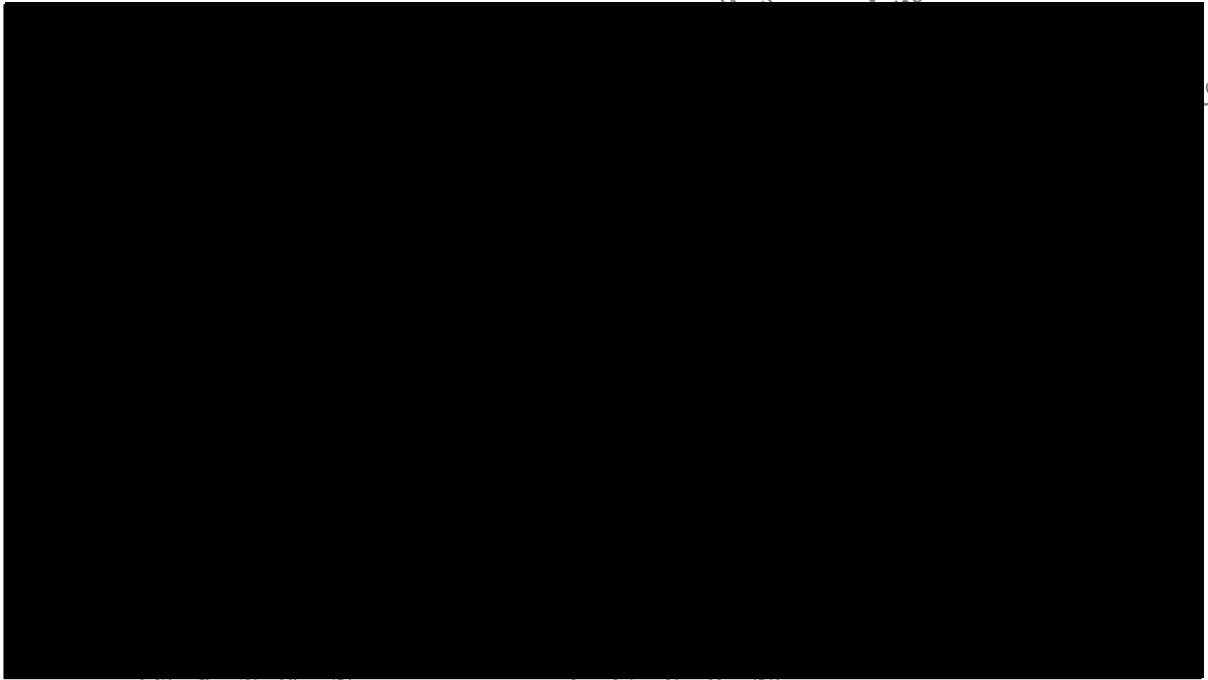
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Certification of Good Laboratory Practice

Statement of Compliance



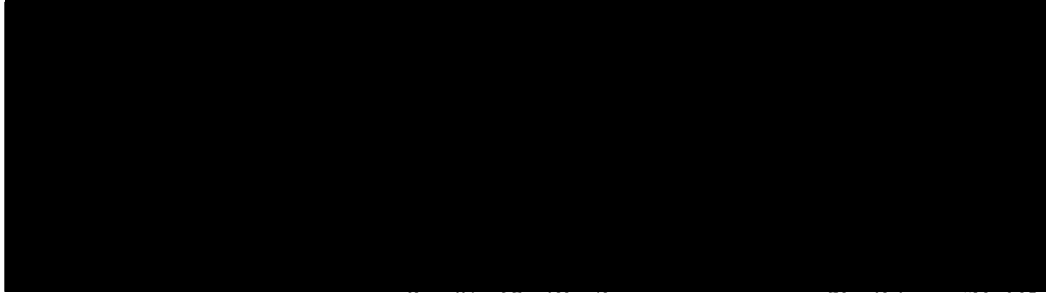
If not otherwise stated, in this report the date format yyyy-mm-dd is used.

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

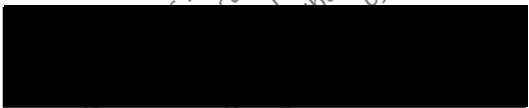
Certification of Authenticity



Inquiries should be directed to:



Bayer CropScience AG
Alfred-Nobel-Str. 50
BCS-D-HS-RA
Building 6610
D-40789 Monheim am Rhein



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

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Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

1 Summary

The purpose of the study was to examine the exposure of honeybees to imidacloprid and its metabolites imidacloprid-hydroxy and imidacloprid-olefine by potential uptake of soil residues of imidacloprid into bee-relevant matrices of winter rape plants in a succeeding crop scenario. This analytical report describes the results of the determination of residues of imidacloprid, imidacloprid-hydroxy, and imidacloprid-olefine in nectar and pollen collected from the flowering winter rape by foraging honeybees.

The study was conducted on the field area of the Bayer CropScience experimental farm Höfchen, close to Burscheid (Nordrhein-Westfalen, Germany). One plot was treated with Manta Plus FS 145.2 G (Fuberidazole + Imazalil + Imidacloprid + Triadimenol FS 145.2 (7.2+8+70+60) G) (spray application and incorporation into soil) and sown with Manta Plus FS 145.2 G dressed winter-wheat seeds. Winter wheat, treated with a similar formulation as used in the treatment part of the study however without imidacloprid, was sown to an additional "imidacloprid-untreated" control plot at the same field. After harvest of the winter wheat plants, in the following summer "imidacloprid"-untreated winter rape seeds were sown on the same treatment and control plots of the study. During the flowering period of the rape, bee tunnels were installed on both fields. Nectar and pollen collected by foraging honeybees were sampled. The samples were directly deep-frozen after sampling and transferred to the laboratory of [REDACTED] on 2009-06-17 where they were stored until analysis at -18°C or below in dark conditions.

Residues of imidacloprid and its metabolites NTN33893-5-hydroxy and NTN33893-olefine in/on nectar and pollen were determined according to method 00537/M002.

Imidacloprid and its metabolites were extracted from the nectar samples with water. The extract was cleaned-up by partition against dichloromethane on a Chromabond XTR™ column. The eluate was evaporated to dryness and diluted with internal standard.

For the sample material pollen, extraction occurred with a mixture of methanol/water 3/1 (v/v). After filtration an aliquot of the extract was concentrated to the aqueous remainder and partitioned against dichloromethane on a Chromabond XTR™ column. A further clean-up by column chromatography on Silica-Gel and subsequent elution with acetonitrile/water was performed. The eluate was evaporated to dryness and diluted with internal standard.

The residues were quantified by reversed phase HPLC with electrospray MS/MS-detection using internal stable labelled standards of imidacloprid and its metabolites NTN33893-5-hydroxy and NTN33893-olefine.

The limit of quantitation (LOQ) for imidacloprid and its metabolites NTN33893-5-hydroxy and NTN33893-olefine was 0.001 mg/kg corresponding to the lowest fortification level of successfully conducted recovery experiments. The limit of detection (LOD) was estimated to be at least 3 times lower (0.0003 mg/kg) than the LOQ, as could be concluded from the linearity response data of the lowest-concentration standards and from the matrix interference observed in control sample chromatograms.

For pollen the individual recovery values for imidacloprid ranged from 73 to 102% with an overall recovery of 88% and with a relative standard deviation (RSD) of 15.4% (n = 4), the individual recovery values for NTN33893-5-hydroxy ranged from 89 to 106% with an overall recovery of 97% and with a RSD of 7.3% (n = 4) and the individual recovery values for NTN33893-olefine ranged from 78 to 105% with an overall recovery of 94% and with a RSD of 12.2% (n = 4).

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

1 Summary (contd)

For nectar the individual recovery values for imidacloprid ranged from 86 to 96% with an overall recovery of 92% and with a RSD of 5.6% (n = 3), the individual recovery values for NTN33893-5-hydroxy ranged from 71 to 81% with an overall recovery of 76% and with a RSD of 6.6% (n = 3) and the individual recovery values for NTN33893-olefine ranged from 96 to 102% with an overall recovery of 99% and with a RSD of 3.1% (n = 3).

All results of the method validation were in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully.

The analytical results for imidacloprid, imidacloprid-olefine and imidacloprid-monohydroxy in nectar and pollen are reported in Table 5 under section 6.3.

2 Organisation and Staff

2.1 Organisation of Project

Sponsor	Bayer CropScience AG Alfred-Nobel-Str. 50 D-40789 Monheim am Rhein
Study Director	[REDACTED]
Analytical Test Site	Bayer CropScience AG BCS-D-ENSA-ETX (since 2009, Feb 1) Alfred-Nobel-Str. 50 D-40789 Monheim am Rhein
Test Facility	Bayer CropScience AG BCS-D-HS-RA (since 2009, Feb 1) Alfred-Nobel-Str. 50 D-40789 Monheim am Rhein

2.2 Responsible Personnel for Residue Analysis

Head of Analytical Test Site	[REDACTED]
PI Analysis and Head of Laboratory	Bayer CropScience AG BCS-D-HS-RA Alfred-Nobel-Str. 50 D-40789 Monheim am Rhein
Technician	[REDACTED]
	Bayer CropScience AG BCS-D-HS-RA D-40789 Monheim am Rhein

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

2.3 Responsible Personnel for the Field Part

Representative Sponsor of the Field Part Bayer CropScience AG
BCS-D-EnSa-ETX
Alfred-Nobel-Str. 50
D-40789 Monheim am Rhein

2.4 Archiving

All raw data pertaining to the analytical phase of this study and the final report of the analytical phase are stored in the central GLP archive of Bayer CropScience AG, Alfred-Nobel-Str. 50, D-40789 Monheim am Rhein for as long as required by GLP principles.

Reserve samples of the reference items are stored in the archives of Bayer CropScience AG, Product Technology-Analytics Frankfurt, Industriepark Höchst, D-65926 Frankfurt, and of Bayer CropScience AG, Research-Product Technology, Isotope Chemistry, Aprather Weg 18a, D-42096 Wuppertal. The test and reference items are stored as long as their quality still guarantees an evaluation.

2.5 Quality Assurance Unit

Bayer CropScience AG
BCS-D-SPQ-GLP/QA
Agricultural Centre Monheim
Alfred-Nobel-Str. 50
D-40789 Monheim am Rhein

2.6 Schedule

Approval of Study by the Study Director	2007-09-18
Start of Analytical Phase (First extraction)	2009-08-10
End of Analytical Phase (Last printout of a chromatogram)	2009-08-24

3 Study Objective

The purpose of the study was to examine the exposure of honeybees to imidacloprid and its metabolites imidacloprid-hydroxy and imidacloprid-olefine by potential uptake of soil residues of imidacloprid into bee-relevant matrices of winter rape plants in a succeeding crop scenario. This analytical report describes the results of the determination of residues of imidacloprid, imidacloprid-hydroxy, and imidacloprid-olefine in nectar and pollen collected from the flowering winter rape by foraging honeybees.

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

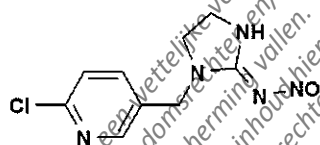
Study No.: E 319 3387-4

4 Compounds

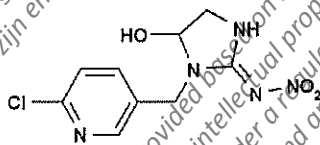
4.1 Reference Items

Name of the Substance
Chemical Name
Empirical Formula
Mol ID**Imidacloprid**
1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine
 $C_9H_{10}ClN_5O_2$
84

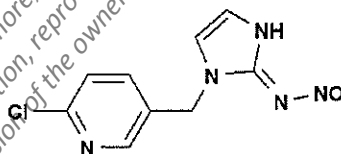
Structural Formula

Molar Mass
Analytical Certificate No.
Batch No.
Purity
Expiration Date
Storage Conditions255.69 g/mol
AZ 15283, dated 2008-10-07
EPE0027068
98.8%
September 2011
 $+5^{\circ}C \pm 5^{\circ}C$ Name of the Substance
Chemical Name
Empirical Formula**Imidacloprid-mono-hydroxy (NTN33893-5-Hydroxy)**
(E)-3-(6-chloro-3-pyridylmethyl)-2-(nitroimino)imidazolidin-4-ol
 $C_9H_{10}ClN_5O_3$

Structural Formula

Molar Mass
Analytical Certificate No.
Batch No.
Expiration Date
Storage Conditions271.67 g/mol
AZ 15434, dated 2008-11-07
KTS10020-5-4
November 2013
 $+5^{\circ}C \pm 5^{\circ}C$ Name of the Substance
Chemical Name
Empirical Formula**Imidacloprid-olefine (NTN33893-Olefine)**
1-(6-chloro-3-pyridylmethyl)-N-nitro-1,3-dihydro-2H-imidazol-2-ylideneamine
 $C_9H_8ClN_5O_2$

Structural Formula

Molar Mass
Analytical Certificate No.
Batch No.
Expiration Date
Storage Conditions253.65 g/mol
AZ 15445, dated 2008-11-11
M24198
October 2013
 $+5^{\circ}C \pm 5^{\circ}C$

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

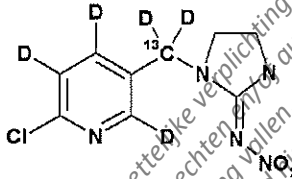
Study No.: E 319 3387-4

4.2 Internal Standards

Name of the Substance
Chemical Name
Empirical Formula

Imidactoprid-d₅-¹³C
[pyridine-d₃, pyridinylmethyl-d₂, ¹³C]
¹³C₈H₅d₅ClN₅O₂

Structural Formula



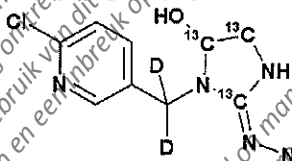
Molar Mass
Analytical Certificate No.
Batch No.
Storage Conditions

258.3 g/mol
BECH 1272-1-1 from 2008-09-02
BECH 1272-1-1
-20°C ± 5°C

Name of the Substance
Chemical Name
Empirical Formula

NTN33893-5-Hydroxy-d₂-¹³C₃
[pyridinylmethyl-d₂, imidazolidinol-¹³C₃]
¹³C₃C₆H₆d₂ClN₅O₃

Structural Formula



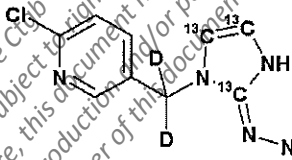
Molar Mass
Analytical Certificate No.
Batch No.
Storage Conditions

276.7 g/mol
BECH 1273-1-4 from 2006-02-14
BECH 1273-1-4
-20°C ± 5°C

Name of the Substance
Chemical Name
Empirical Formula

NTN33893-Olefine-d₂-¹³C₃
[pyridinylmethyl-d₂, dihydroimidazole-¹³C₃]
¹³C₃C₆H₅d₂ClN₅O₂

Structural Formula



Molar Mass
Analytical Certificate No.
Batch No.
Storage Conditions

258.6 g/mol
BECH 1274-1-2 from 2006-02-14
BECH 1274-1-2
-20°C ± 5°C

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

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5 Sample Material and Sample Preparation

The study was conducted on the field area of the Bayer CropScience experimental farm Höfchen, close to Burscheid (Nordrhein-Westfalen, Germany). One plot was treated with Manta Plus FS 145.2 G (Fuberidazole + Imazalil + Imidacloprid + Triadimenol FS 145.2 (7.2+8+70+60) G) (spray application and incorporation into soil) and sown with Manta Plus FS 145.2 G dressed winter-wheat seeds. Winter wheat, treated with a similar formulation as used in the treatment part of the study however without imidacloprid, was sown to an additional "imidacloprid-untreated" control plot at the same field. After harvest of the winter wheat plants, in the following summer "imidacloprid"-untreated winter rape seeds were sown on the same treatment and control plots of the study. During the flowering period of the rape, bee tunnels were installed on both fields. Nectar and pollen collected by foraging honeybees were sampled. The samples were directly deep-frozen after sampling and transferred to the laboratory of [REDACTED] on 2009-06-17 where they were stored until analysis at -18°C or below in dark conditions. An overview of the samples generated is shown in Table 1.

Table 1: Summary of Field Samples

Sample Number	Sample Name	Sample Material	Treated/Control Plot [T/C]	Sample Weight [g]	
002	Pollen C2	Pollen	C	0.0451	
004	Pollen C4		C	0.5655	
006	Pollen C6		C	0.8363	
008	Pollen C8		C	0.5239	
001	Pollen T1		T	0.1061	
003	Pollen T3		T	0.4394	
005	Pollen T5		T	0.8202	
007	Pollen T7		T	0.5164	
002	Nectar C2		Nectar	C	1.7717
004	Nectar C4			C	1.4733
006	Nectar C6			C	1.9816
008	Nectar C8			C	2.0007
001	Nectar T1			T	1.6078
003	Nectar T3			T	1.4880
005	Nectar T5	T		1.6905	
007	Nectar T7	T	1.5728		

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
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6 Residue Analyses

6.1 Analytical Method

Method No.	00537
Author	[REDACTED]
Citation	MR-551/98, dated on 2001-12-31
Modification	M002
Author	[REDACTED]
Citation	MR-06/144, dated on 2006-11-02

Residues of imidacloprid and its metabolites NTN33893-5-hydroxy and NTN33893-olefine in/on nectar and pollen were determined according to method 00537/M002.

Imidacloprid and its metabolites were extracted from the nectar samples with water. The extract was cleaned-up by partition against dichloromethane on a Chromabond XTR™ column. The eluate was evaporated to dryness and diluted with internal standard.

For the sample material pollen, extraction occurred with a mixture of methanol/water 3/1 (v/v). After filtration an aliquot of the extract was concentrated to the aqueous remainder and partitioned against dichloromethane on a Chromabond XTR™ column. A further clean-up by column chromatography on Silica-Gel and subsequent elution with acetonitrile/water was performed. The eluate was evaporated to dryness and diluted with internal standard.

The residues were quantified by reversed phase HPLC with electrospray MS/MS-detection using internal stable labelled standards of imidacloprid and its metabolites NTN33893-5-hydroxy and NTN33893-olefine.

Detailed information of the HPLC and MS/MS conditions are given in Appendix 2.

The limit of quantitation (LOQ) for imidacloprid and its metabolites NTN33893-5-hydroxy and NTN33893-olefine was 0.001 mg/kg corresponding to the lowest fortification level of successfully conducted recovery experiments. The limit of detection (LOD) was estimated to be at least 3 times lower (0.0003 mg/kg) than the LOQ, as could be concluded from the linearity response data of the lowest-concentration standards and from the matrix interference observed in control sample chromatograms. Representative linearity plots are given in Appendix 3.

Data on method validation are given in Chapter 6.2.

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

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6.2 Method Validation

The analytical method was validated by running concurrent recoveries at the LOQ and higher residues levels. Therefore control samples were spiked with a mixture of imidacloprid and its metabolites NTN33893-5-hydroxy and NTN33893-olefine. Fortification levels and recovery data are given in Table 2 to Table 4.

Table 2: Recovery Data for Imidacloprid in/on Pollen and Nectar.
FL: Fortification Level, RSD: Relative Standard Deviation, LOQ: Practical Limit of Quantitation.

Sample Material	Fortification Level [mg/kg]	Recoveries - Single Values [%]			Mean [%]	RSD [%]
Pollen	0.001	80	73	96	83	14.2
	0.10	102			--	--
	Overall Mean and RSD [%]				88	15.4
Nectar	0.001	93	86	96	92	5.6
	Overall Mean and RSD [%]				92	5.6

Note: Fortified, determined and calculated as imidacloprid.

Table 3: Recovery Data for NTN33893-5-hydroxy in/on Pollen and Nectar.
FL: Fortification Level, RSD: Relative Standard Deviation, LOQ: Practical Limit of Quantitation.

Sample Material	Fortification Level [mg/kg]	Recoveries - Single Values [%]			Mean [%]	RSD [%]
Pollen	0.001	95	97	106	99	5.9
	0.10	89			--	--
	Overall Mean and RSD [%]				97	7.3
Nectar	0.001	72	71	81	76	6.6
	Overall Mean and RSD [%]				76	6.6

Note: Fortified, determined and calculated as NTN33893-5-hydroxy.

Table 4: Recovery Data for NTN33893-olefine in/on Pollen and Nectar.
FL: Fortification Level, RSD: Relative Standard Deviation, LOQ: Practical Limit of Quantitation.

Sample Material	Fortification Level [mg/kg]	Recoveries - Single Values [%]			Mean [%]	RSD [%]
Pollen	0.001	98	105	78	94	15.0
	0.10	96			--	--
	Overall Mean and RSD [%]				94	12.2
Nectar	0.001	100	96	102	99	3.1
	Overall Mean and RSD [%]				99	3.1

Note: Fortified, determined and calculated as NTN33893-olefine.

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
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6.2 Method Validation (contd)

For pollen the individual recovery values for imidacloprid ranged from 73 to 102% with an overall recovery of 88% and with a relative standard deviation (RSD) of 15.4% (n = 4), the individual recovery values for NTN33893-5-hydroxy ranged from 89 to 106% with an overall recovery of 97% and with a RSD of 7.3% (n = 4) and the individual recovery values for NTN33893-olefine ranged from 78 to 105% with an overall recovery of 94% and with a RSD of 12.2% (n = 4).

For nectar the individual recovery values for imidacloprid ranged from 86 to 96% with an overall recovery of 92% and with a RSD of 5.6% (n = 3), the individual recovery values for NTN33893-5-hydroxy ranged from 71 to 81% with an overall recovery of 76% and with a RSD of 6.6% (n = 3) and the individual recovery values for NTN33893-olefine ranged from 96 to 102% with an overall recovery of 99% and with a RSD of 3.1% (n = 3).

All results of the method validation were in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully.

Representative chromatograms are included in Appendix 1.

6.3 Results

Table 5 shows the results obtained for the nectar and pollen samples.

Table 5: Analytical Results for Imidacloprid, NTN33893-5-hydroxy and NTN33893-olefine in Nectar and Pollen (LOQ = 0.001 mg/kg, LOD = 0.0003 mg/kg)

Sample Number	Sample Name	Sample Material	Treated / Control plot (T/C)	Residue [mg/kg]		
				Imidacloprid	NTN33893-5-hydroxy	NTN33893-olefine
002	Pollen C2	Pollen	C	< LOD	0.004	< LOD
004	Pollen C4		C	< LOD	< LOD	< LOD
006	Pollen C6		C	< LOD	< LOD	< LOD
008	Pollen C8		C	< LOD	< LOD	< LOD
001	Pollen T1		T	0.0003	< LOD	< LOD
003	Pollen T3		T	< LOD	< LOD	< LOD
005	Pollen T5		T	0.0003	< LOD	< LOD
007	Pollen T7		T	< LOD	< LOD	< LOD
002	Nectar C2	Nectar	C	< LOD	< LOD	< LOD
004	Nectar C4		C	< LOD	< LOD	< LOD
006	Nectar C6		C	< LOD	< LOD	< LOD
008	Nectar C8		C	< LOD	< LOD	< LOD
001	Nectar T1		T	< LOD	< LOD	< LOD
003	Nectar T3		T	< LOD	< LOD	< LOD
005	Nectar T5		T	< LOD	< LOD	< LOD
007	Nectar T7		T	< LOD	< LOD	< LOD

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

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7 Evaluation and Discussion

For pollen the individual recovery values for imidacloprid ranged from 73 to 102% with an overall recovery of 88% and with a relative standard deviation (RSD) of 15.4% (n = 4), the individual recovery values for NTN33893-5-hydroxy ranged from 89 to 106% with an overall recovery of 97% and with a RSD of 7.3% (n = 4) and the individual recovery values for NTN33893-olefine ranged from 78 to 105% with an overall recovery of 94% and with a RSD of 12.2% (n = 4).

For nectar the individual recovery values for imidacloprid ranged from 86 to 96% with an overall recovery of 92% and with a RSD of 5.6% (n = 3), the individual recovery values for NTN33893-5-hydroxy ranged from 71 to 81% with an overall recovery of 76% and with a RSD of 6.6% (n = 3) and the individual recovery values for NTN33893-olefine ranged from 96 to 102% with an overall recovery of 99% and with a RSD of 3.1% (n = 3).

All results of the method validation were in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully.

The analytical results for imidacloprid, imidacloprid-olefine and imidacloprid-mono-hydroxy in nectar and pollen are reported in Table 5 under section 6.3.

8 References

- [1] Residue Analytical Method for the Determination of Residues of Imidacloprid, Hydroxy-Metabolite and Olefine-Metabolite in Nectar, Honey, Rape Flower, Rape Pollen and Bee Samples by HPLC with Electrospray MS/MS-detection; Method No.: 00537; Report No MR-551/98; Author: [REDACTED]; Study Completion Date: 2001-12-31.
- [2] Modification M002 of the Analytical Method 00537 for the Determination of Residues of Imidacloprid, Hydroxy-Metabolite and Olefine-Metabolite in Nectar/Honey, Flowers/Blossoms, Pollen, Leaves and Insect Samples by HPLC with Electrospray MS/MS-detection; Method No.: 00537/M002; Report No MR-06/144; Author: [REDACTED]; Study Completion Date: 2006-11-02.

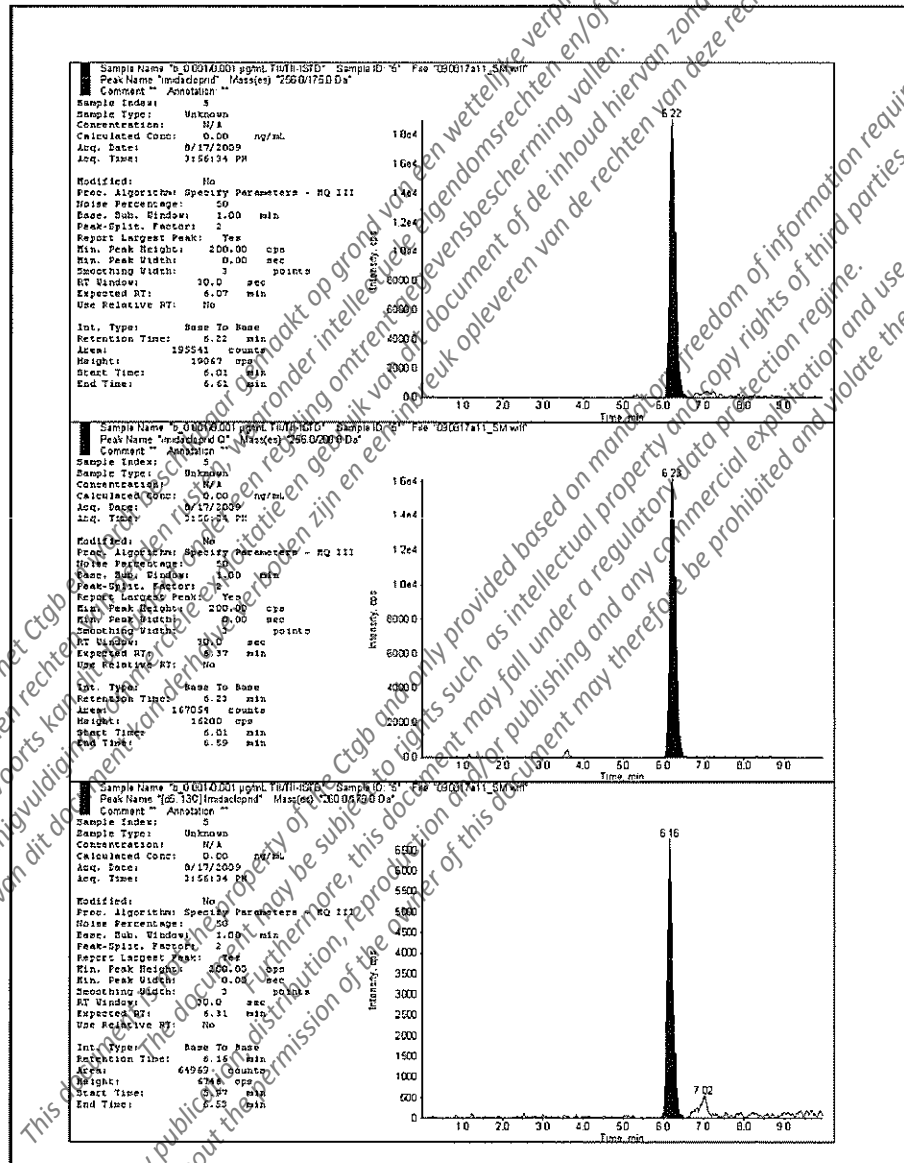
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms

Figure 1 : Standard Solution Imidacloprid 0.001 mg/L and Imidacloprid-d₅¹³C 0.001 mg/L in Solvent
Top: Imidacloprid, 1st MRM
Middle: Imidacloprid, 2nd MRM
Bottom: Internal Standard Imidacloprid-d₅¹³C



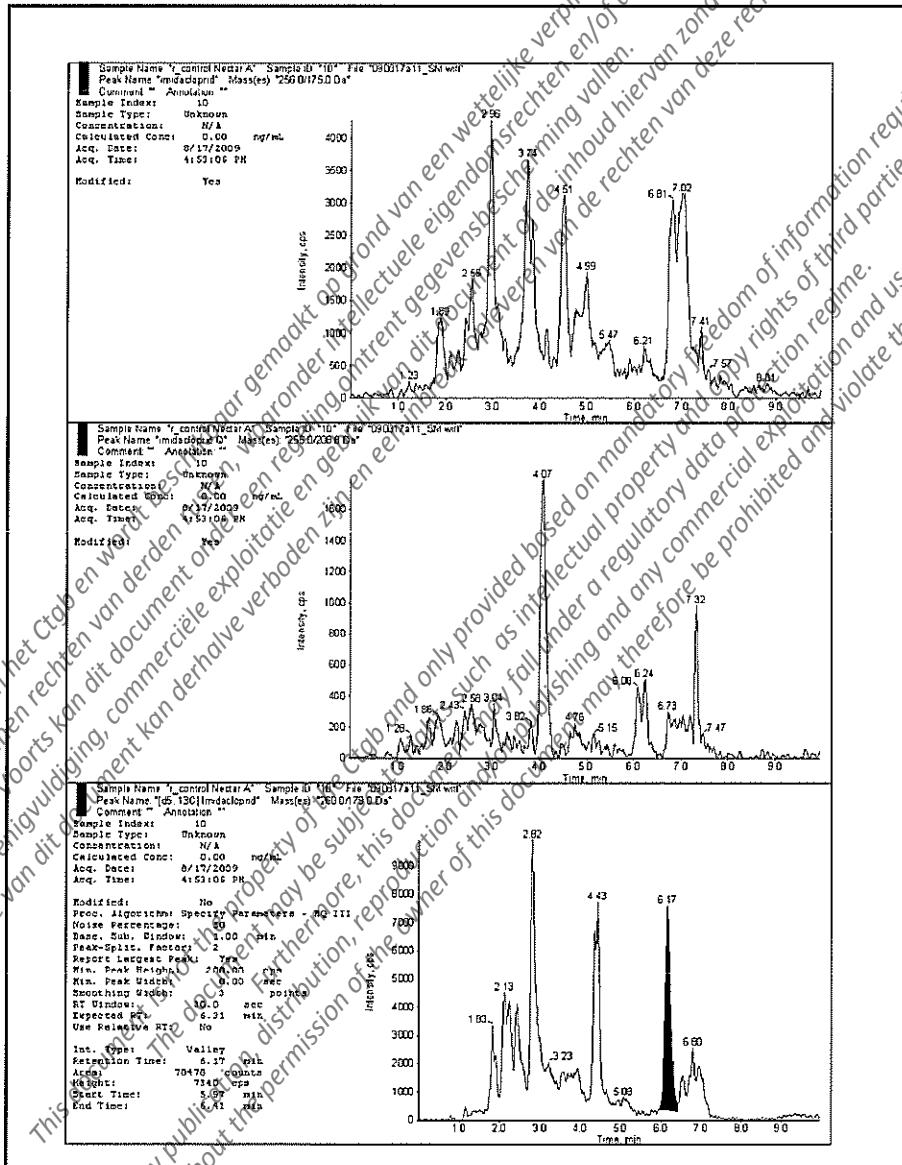
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (contd)

Figure 2 : Control Sample Nectar
Top: Imidacloprid, 1st MRM
Middle: Imidacloprid, 2nd MRM
Bottom: Internal Standard Imidacloprid-d₅¹³C



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

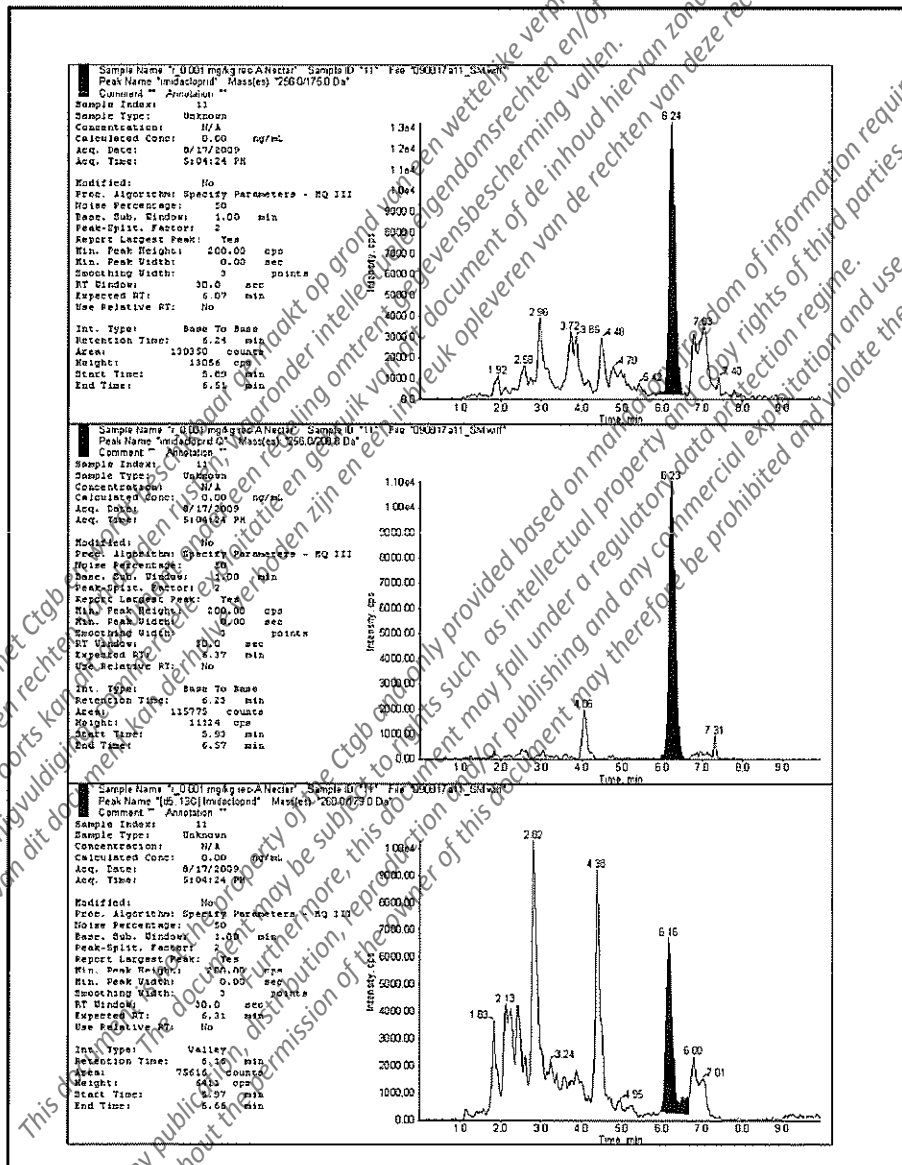
Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (contd)

Figure 3 : Recovery Sample 0.001 mg/kg Imidacloprid in Nectar

Top: Imidacloprid, 1st MRM
Middle: Imidacloprid, 2nd MRM
Bottom: Internal Standard Imidacloprid-d₅¹³C



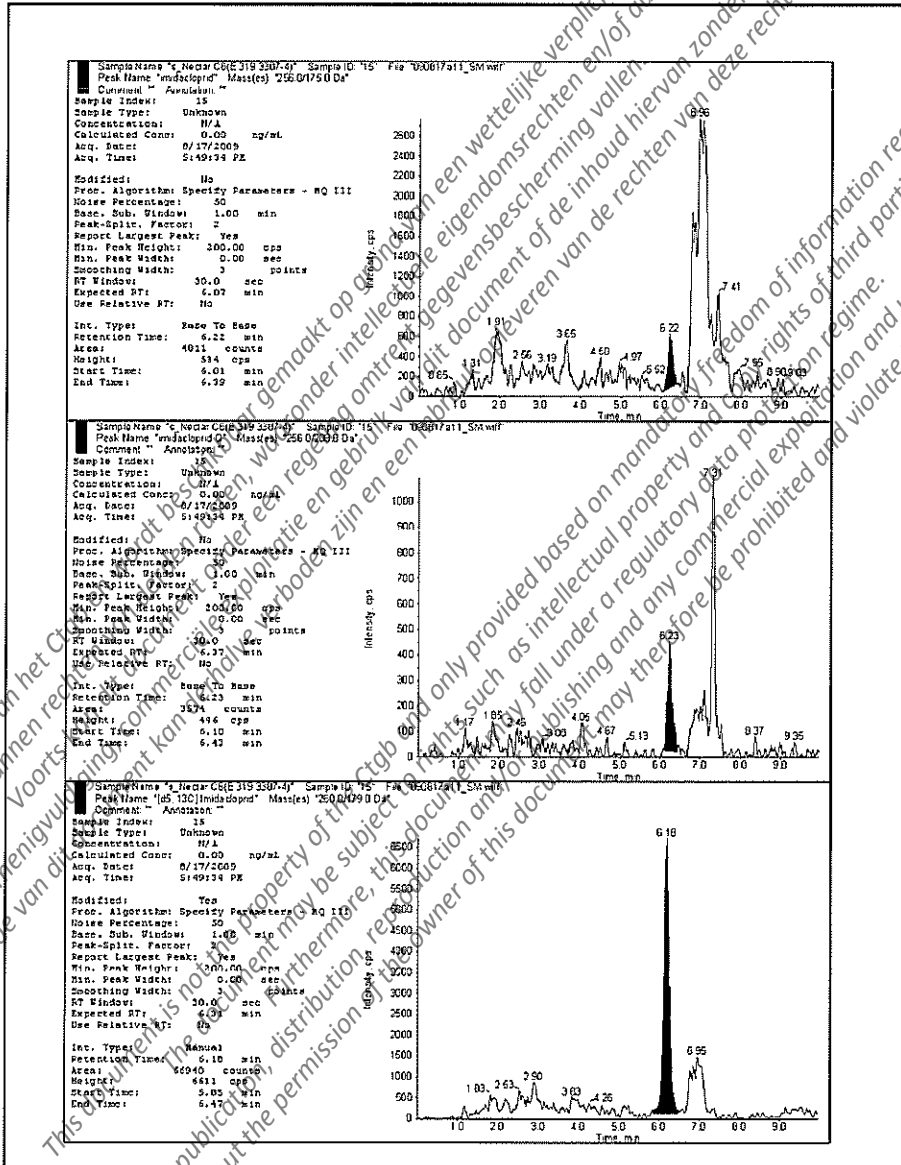
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
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Appendix 1: Representative Chromatograms (cont'd)

Figure 4 : Control ("Imidacloprid-untreated") Sample Nectar C6
Top: Imidacloprid, 1st MRM
Middle: Imidacloprid, 2nd MRM
Bottom: Internal Standard Imidacloprid-d₅¹³C



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

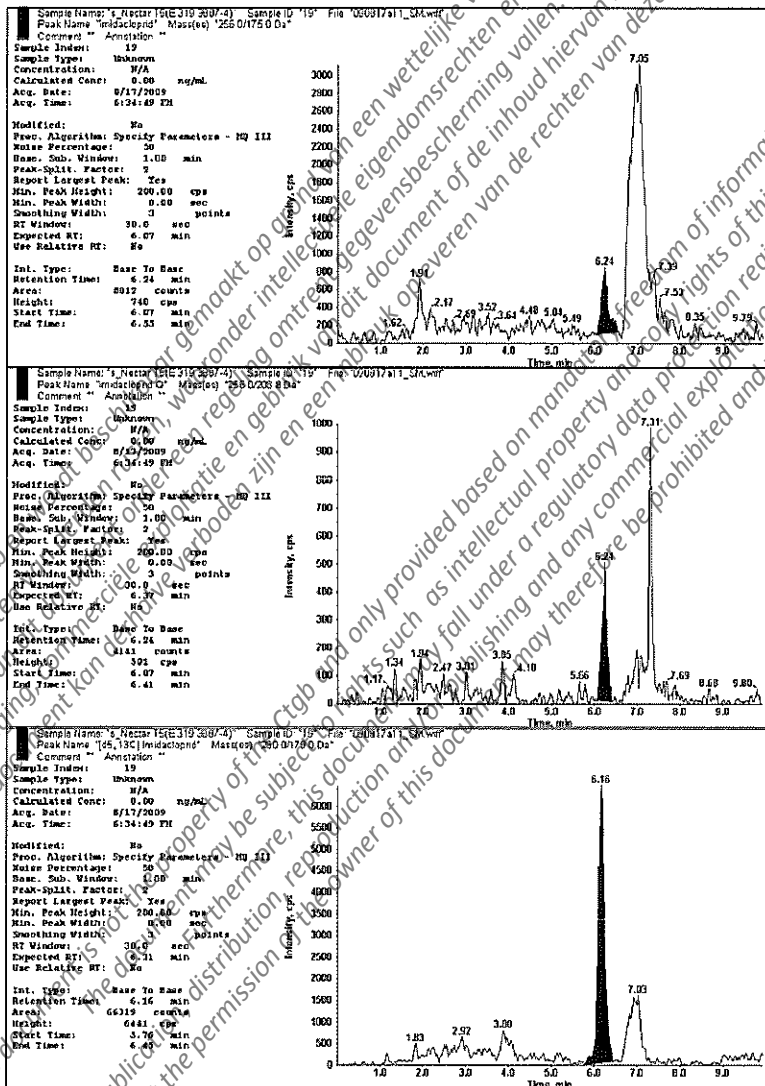
Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (contd)

Figure 5 : Treated Sample Nectar T5

Top: Imidacloprid, 1st MRM
Middle: Imidacloprid, 2nd MRM
Bottom: Internal Standard Imidacloprid-d₅ ¹³C



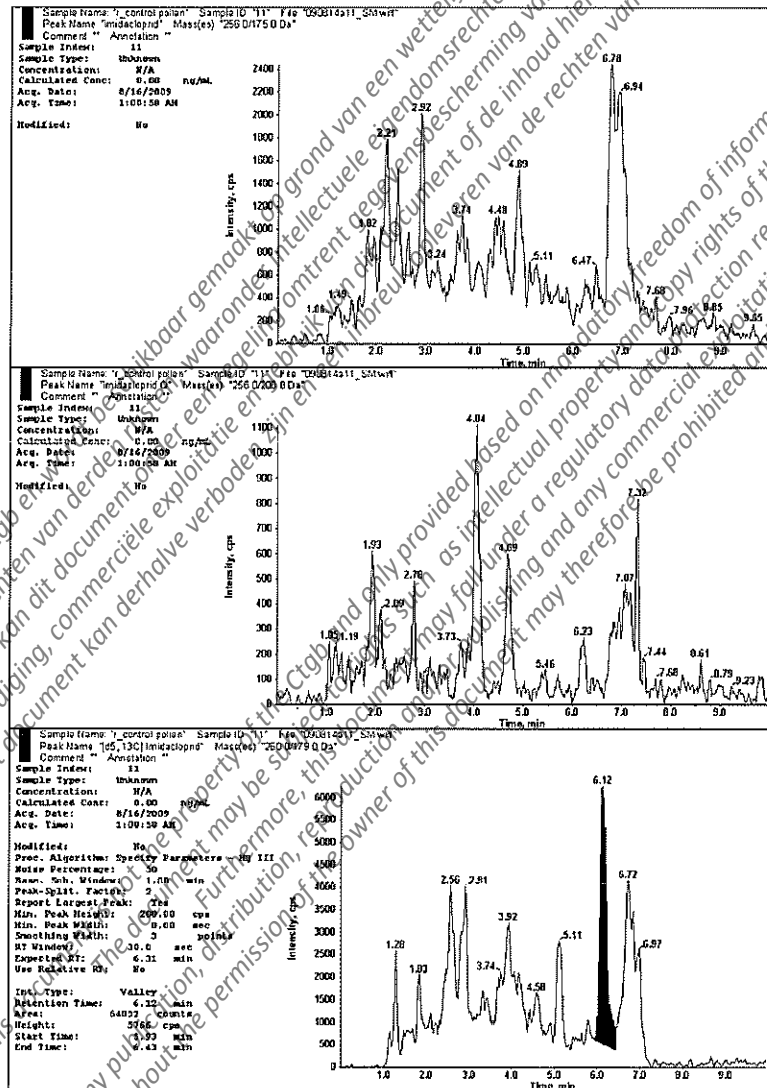
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (contd)

Figure 6 : Control Sample Pollen
Top: Imidacloprid, 1st MRM
Middle: Imidacloprid, 2nd MRM
Bottom: Internal Standard Imidacloprid-d₅¹³C



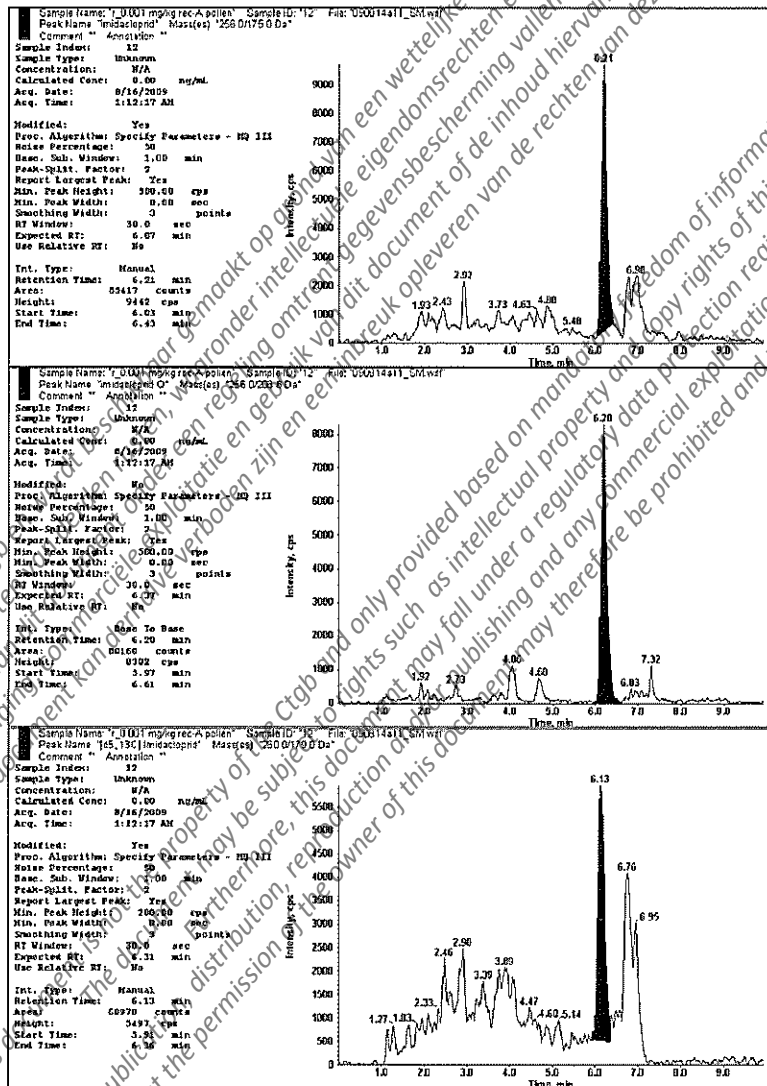
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (contd)

Figure 7 : Recovery Sample 0.001 mg/kg Imidacloprid in Pollen
Top: Imidacloprid, 1st MRM
Middle: Imidacloprid, 2nd MRM
Bottom: Internal Standard Imidacloprid-d₅ ¹³C



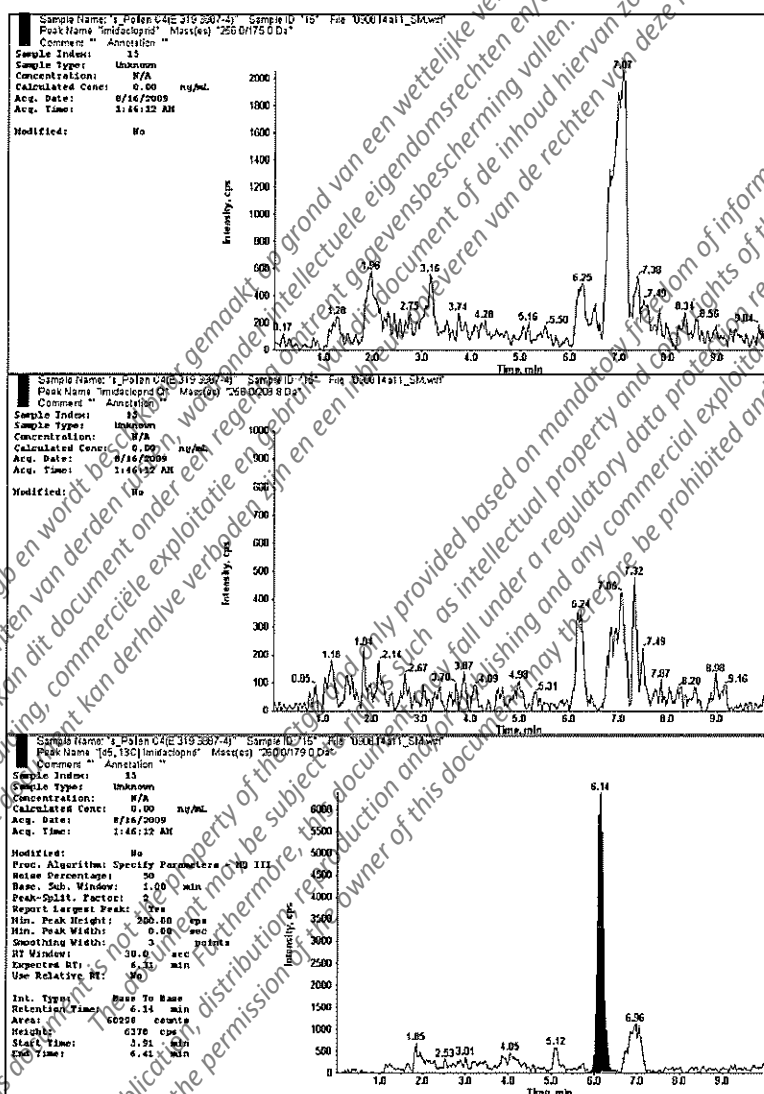
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1:
Representative Chromatograms (contd)

Figure 8 : Control ("Imidacloprid-untreated") Sample Pollen C4
Top: Imidacloprid, 1st MRM
Middle: Imidacloprid, 2nd MRM
Bottom: Internal Standard Imidacloprid-d₅¹³C



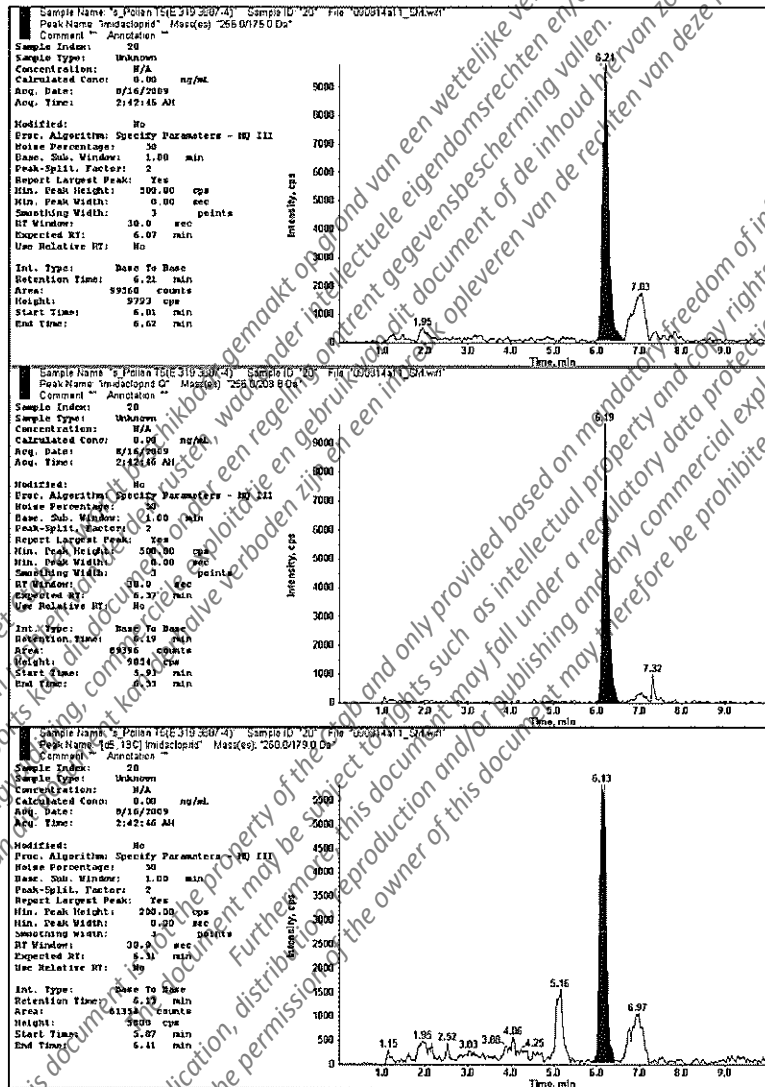
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
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Appendix 1: Representative Chromatograms (contd)

Figure 9 : Treated Sample Pollen T5
Top: Imidacloprid, 1st MRM
Middle: Imidacloprid, 2nd MRM
Bottom: Internal Standard Imidacloprid-d₅¹³C



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

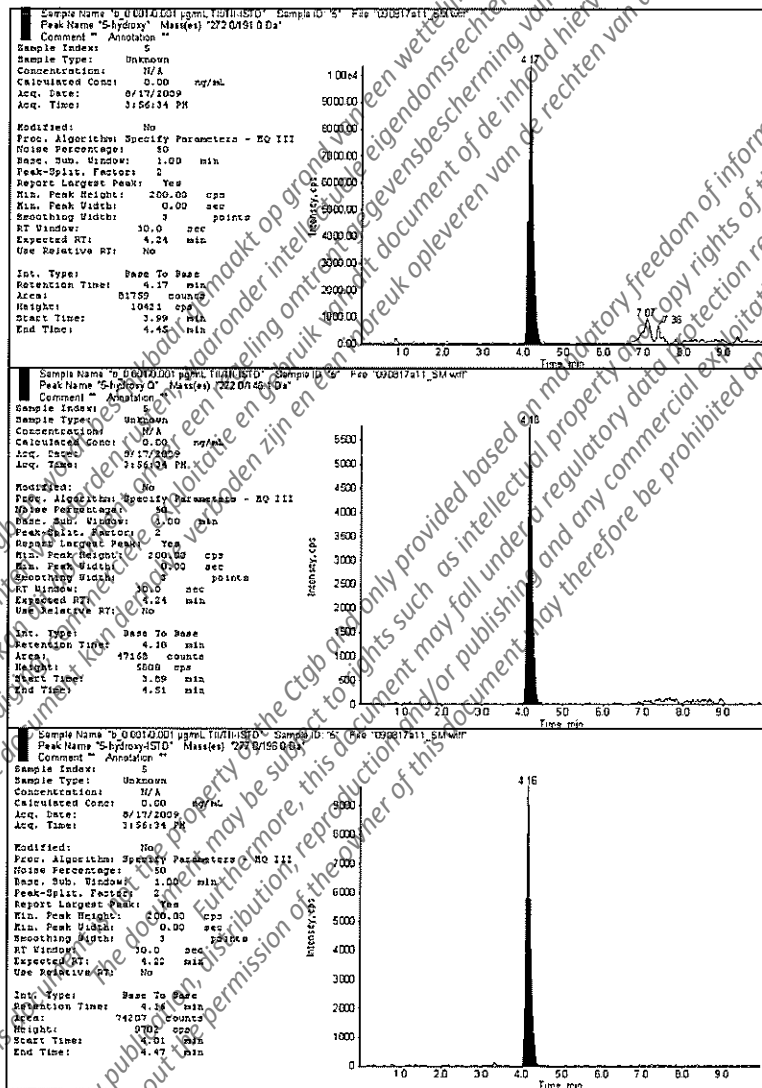
Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1:
Representative Chromatograms (contd)

Figure 10 : Standard Solution NTN33893-5-hydroxy 0.001 mg/L and NTN33893-5-hydroxy-d₂¹³C³ 0.001 mg/L in Solvent

Top: NTN33893-5-hydroxy, 1st MRM
Middle: NTN33893-5-hydroxy, 2nd MRM
Bottom: Internal Standard NTN33893-5-hydroxy-d₂¹³C³



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
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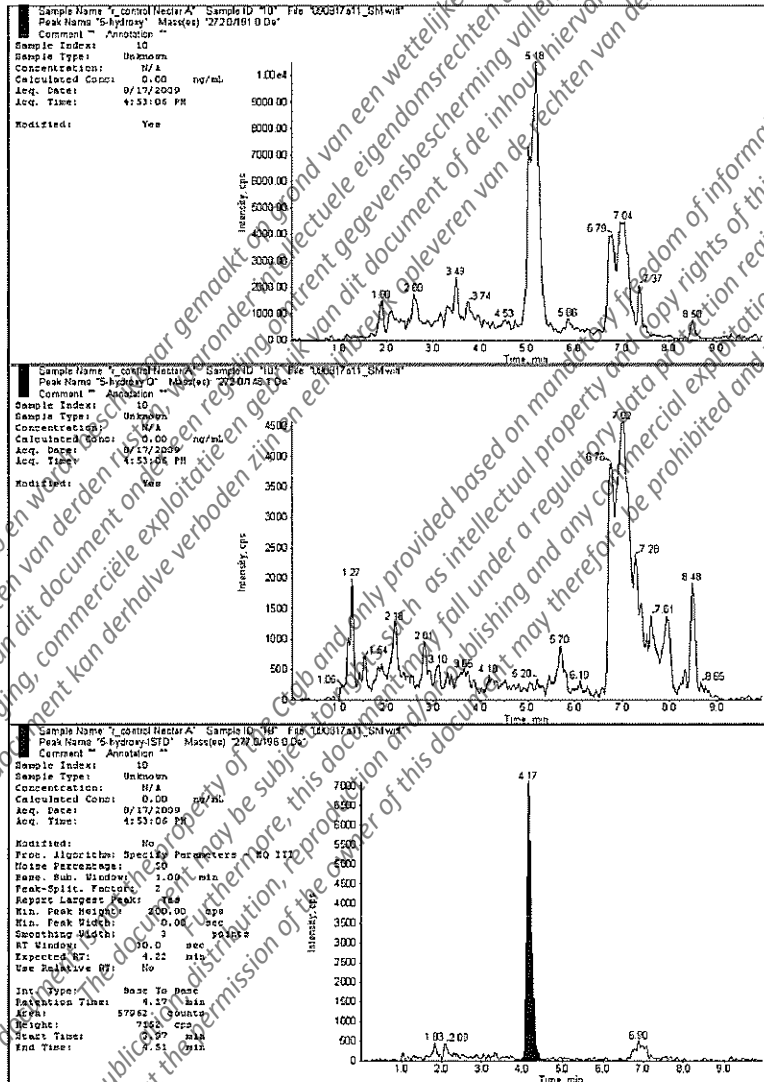
Appendix 1: Representative Chromatograms (cont'd)

Figure 11 : Control Sample Nectar

Top: NTN33893-5-hydroxy, 1st MRM

Middle: NTN33893-5-hydroxy, 2nd MRM

Bottom: Internal Standard NTN33893-5-hydroxy, 1st MRM



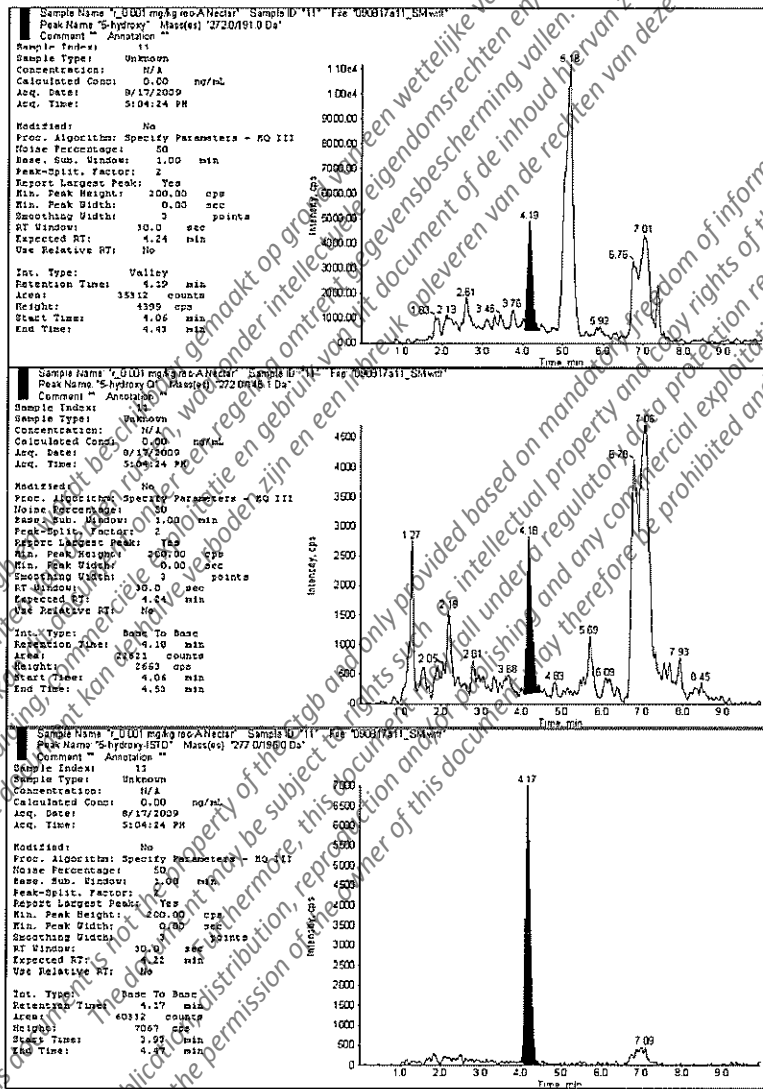
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (contd)

Figure 12 : Recovery Sample 0.001 mg/kg NTN33893-5-hydroxy in Nectar
Top: NTN33893-5-hydroxy, 1st MRM
Middle: NTN33893-5-hydroxy, 2nd MRM
Bottom: Internal Standard NTN33893-5-hydroxy-d₂ C₃



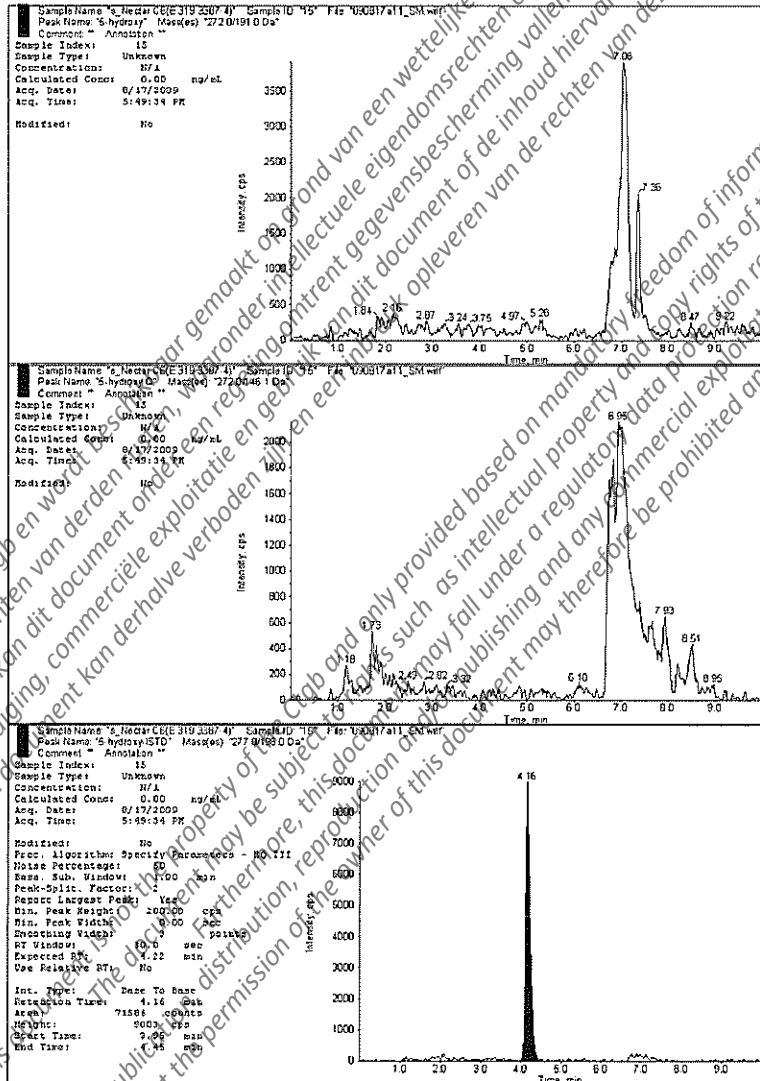
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (contd)

Figure 13 : Control ("Imidacloprid-untreated") Sample Nectar C6
Top: NTN33893-5-hydroxy, 1st MRM
Middle: NTN33893-5-hydroxy, 2nd MRM
Bottom: Internal Standard NTN33893-5-hydroxy-d₂



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

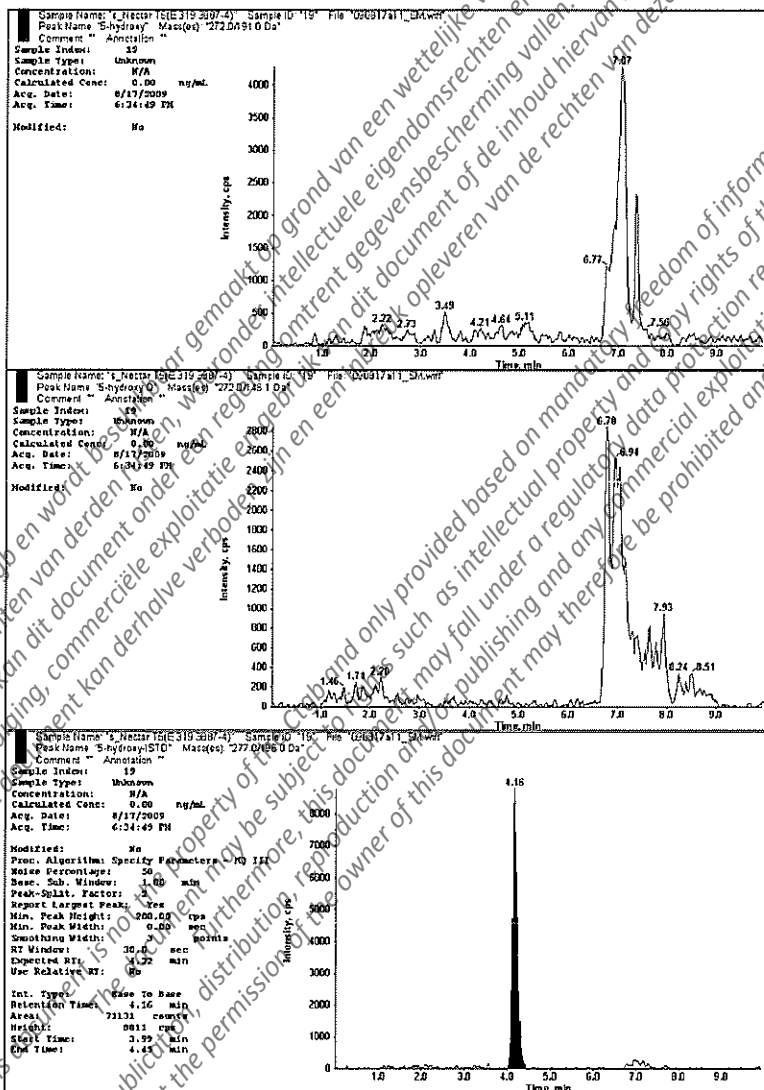
Appendix 1: Representative Chromatograms (contd)

Figure 14 : Treated Sample Nectar T5

Top: NTN33893-5-hydroxy, 1st MRM

Middle: NTN33893-5-hydroxy, 2nd MRM

Bottom: Internal Standard NTN33893-5-hydroxy-d₂-C₃



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

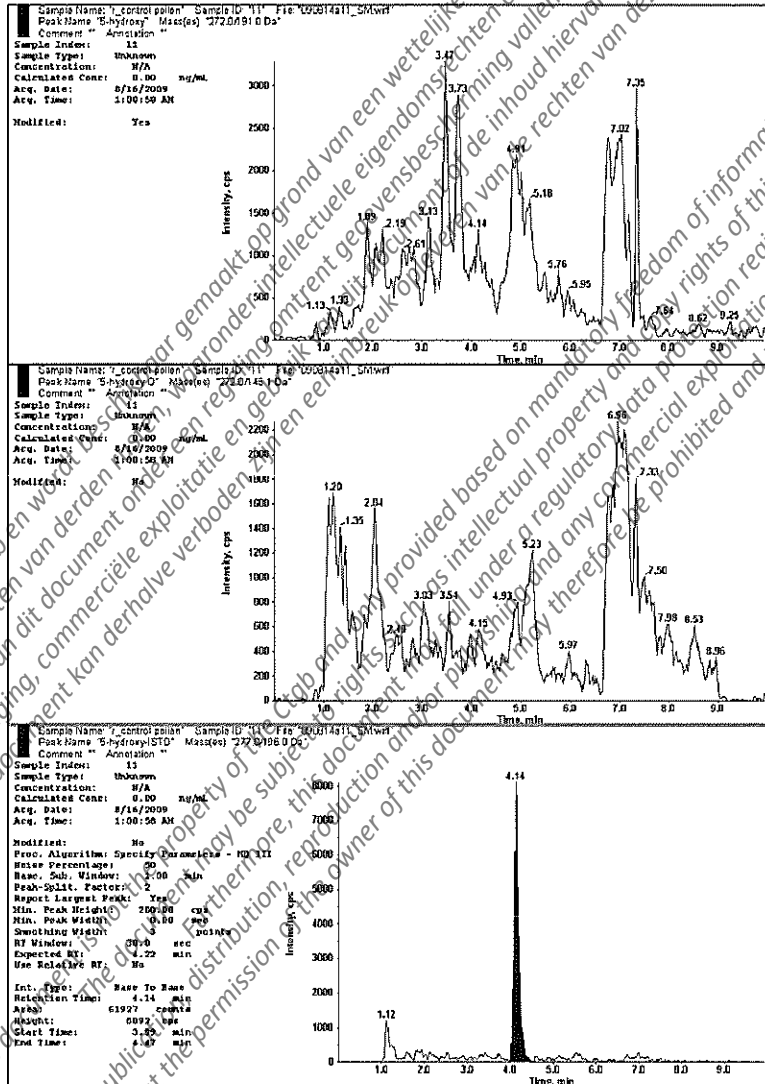
Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (cont'd)

Figure 15 : Control Sample Pollen

Top: NTN33893-5-hydroxy, 1st MRM
Middle: NTN33893-5-hydroxy, 2nd MRM
Bottom: Internal Standard NTN33893-5-hydroxy, 1st MRM



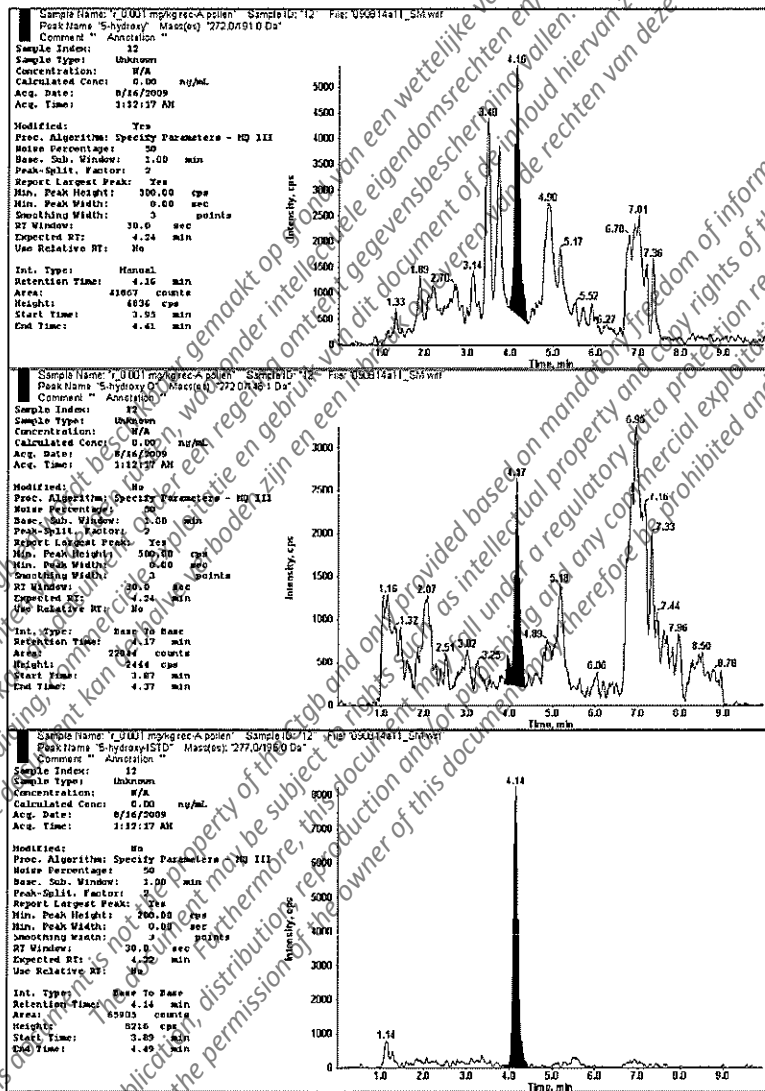
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1:
Representative Chromatograms (contd)

Figure 16 : Recovery Sample 0.001 mg/kg NTN33893-5-hydroxy in Pollen
Top: NTN33893-5-hydroxy, 1st MRM
Middle: NTN33893-5-hydroxy, 2nd MRM
Bottom: Internal Standard NTN33893-5-hydroxy-d₂



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

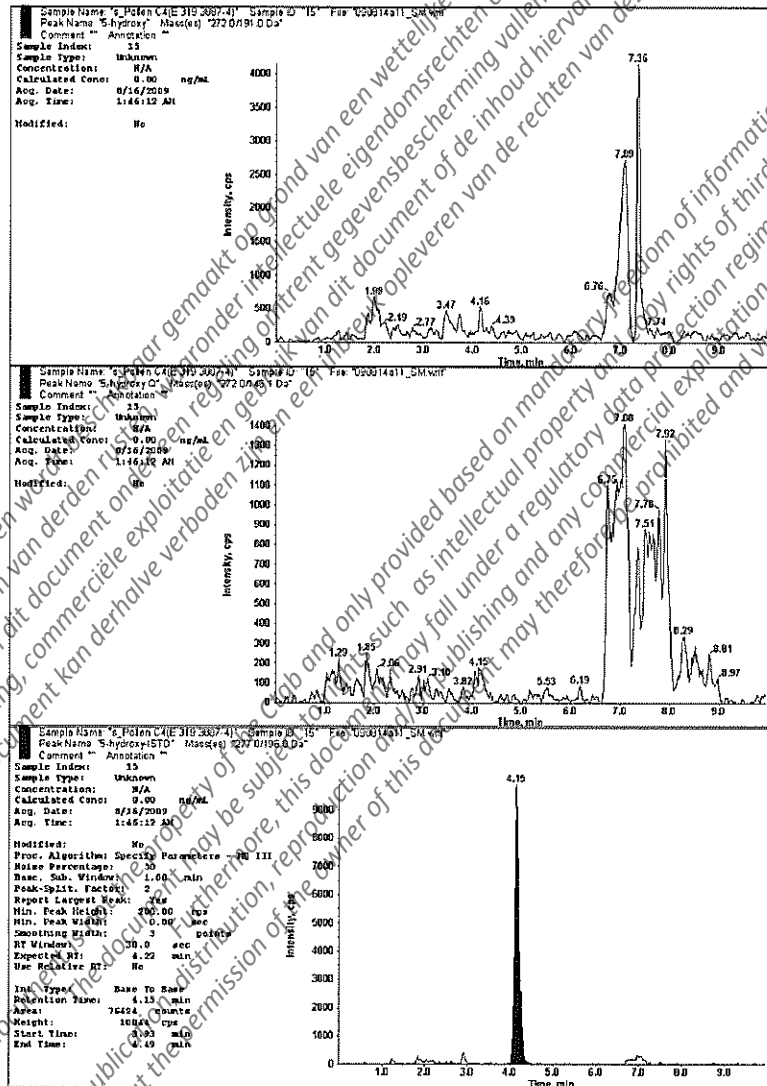
Appendix 1: Representative Chromatograms (confd)

Figure 17 : Control ("Imidacloprid-untreated") Sample Pollen C4

Top: NTN33893-5-hydroxy, 1st MRM

Middle: NTN33893-5-hydroxy, 2nd MRM

Bottom: Internal Standard NTN33893-5-hydroxy, 1st MRM



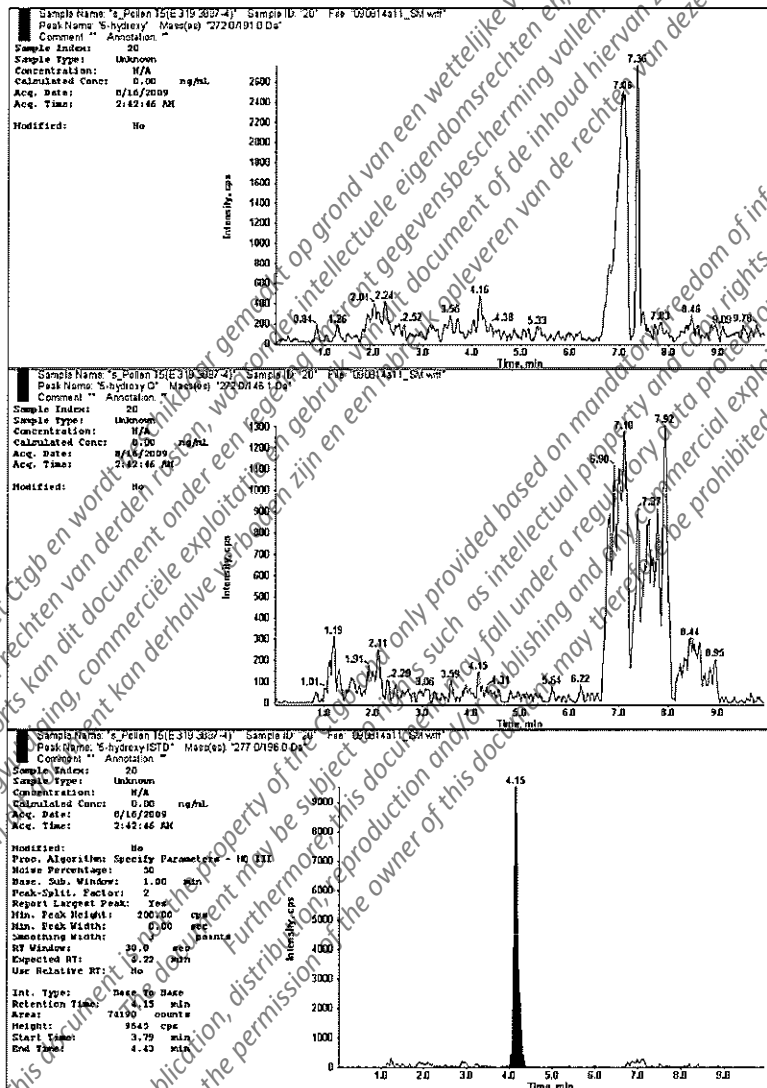
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (contd)

Figure 18 : Treated Sample Pollen T5
Top: NTN33893-5-hydroxy, 1st MRM
Middle: NTN33893-5-hydroxy, 2nd MRM
Bottom: Internal Standard NTN33893-5-hydroxy-D₂



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

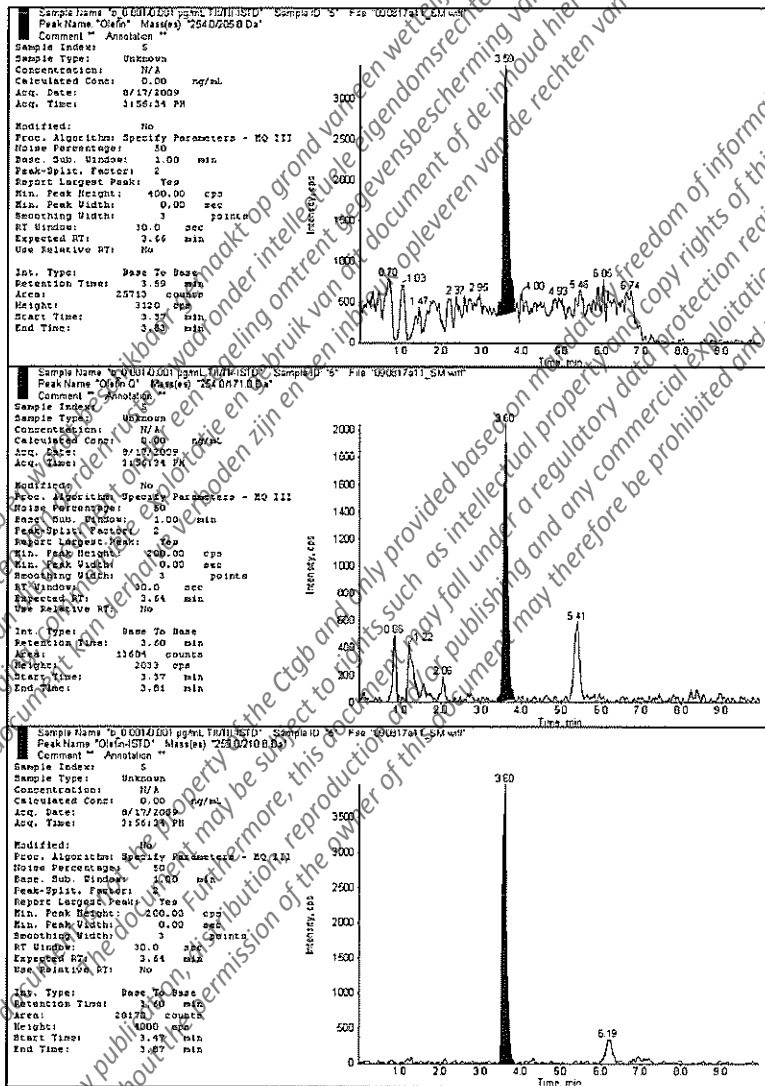
Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1:
Representative Chromatograms (contd)

Figure 19 : Standard Solution NTN33893-olefine 0.001 mg/L and NTN33893-olefine-¹³C₁₅ 0.001 mg/L in Solvent

Top: NTN33893-olefine, 1st MRM
Middle: NTN33893-olefine, 2nd MRM
Bottom: Internal Standard NTN33893-olefine-¹³C₁₅



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

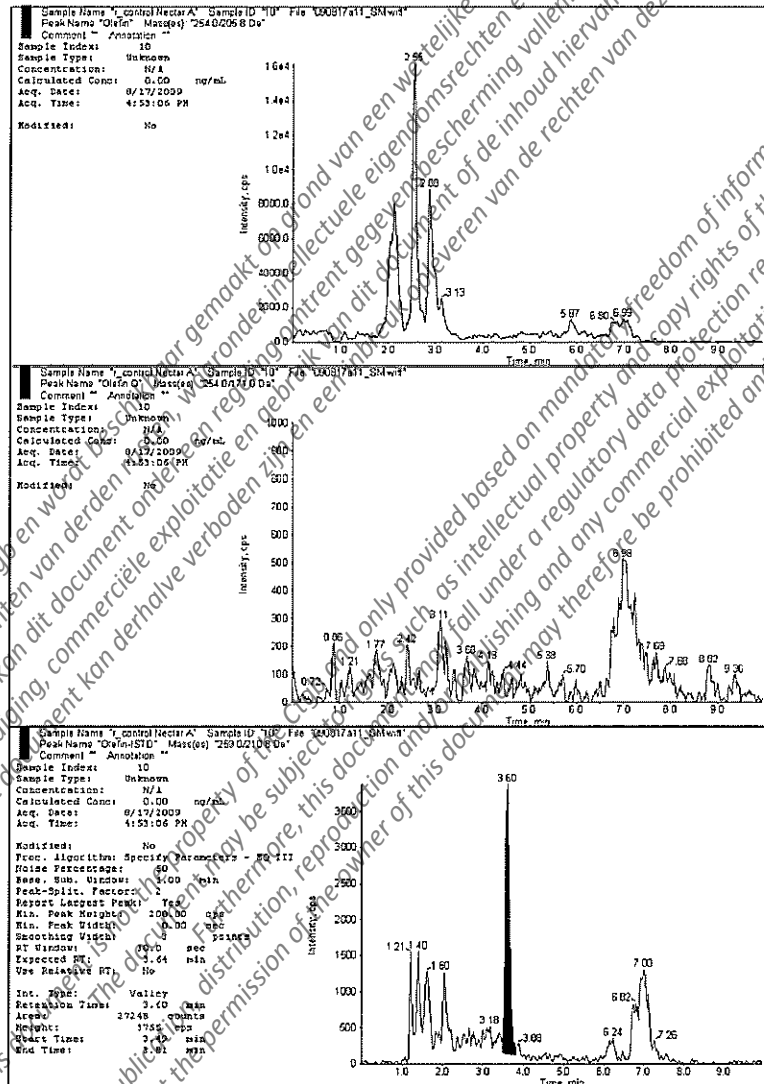
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Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (contd)

Figure 20 : Control Sample Nectar

Top: NTN33893-olefine, 1st MRM
Middle: NTN33893-olefine, 2nd MRM
Bottom: Internal Standard NTN33893-olefine-¹³C¹⁵N



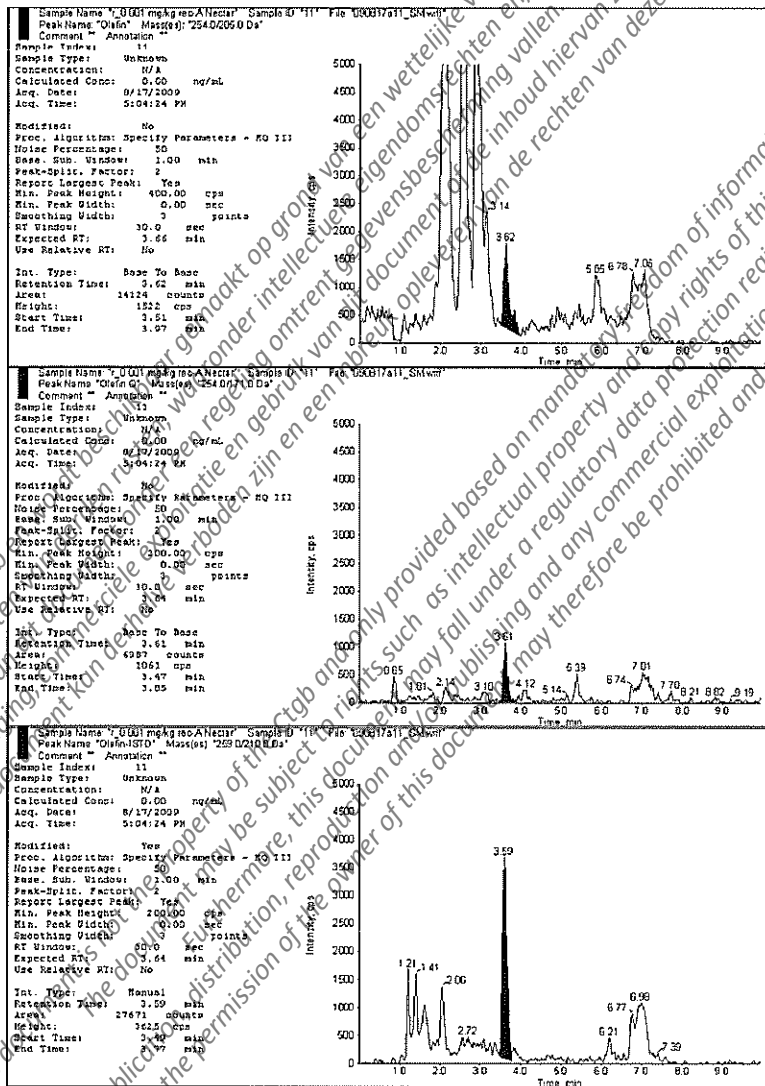
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
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Appendix 1: Representative Chromatograms (cont'd)

Figure 21 : Recovery Sample 0.001 mg/kg NTN33893-olefine in Nectar
Top: NTN33893-olefine, 1st MRM
Middle: NTN33893-olefine, 2nd MRM
Bottom: Internal Standard NTN33893-olefine-¹³C¹⁶N



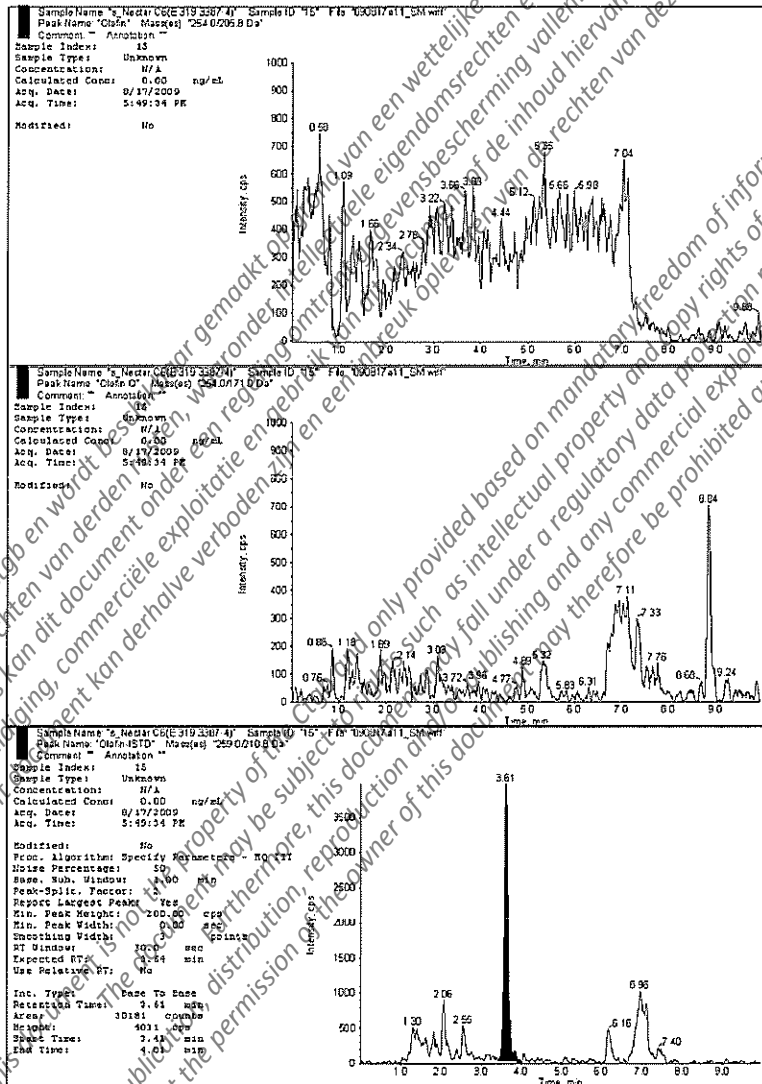
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1:
Representative Chromatograms (contd)

Figure 22 : Control (¹⁴C-Imidacloprid-untreated) Sample Nectar C6
Top: NTN33893-olefine, 1st MRM
Middle: NTN33893-olefine, 2nd MRM
Bottom: Internal Standard NTN33893-olefine-¹³C¹⁵N



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

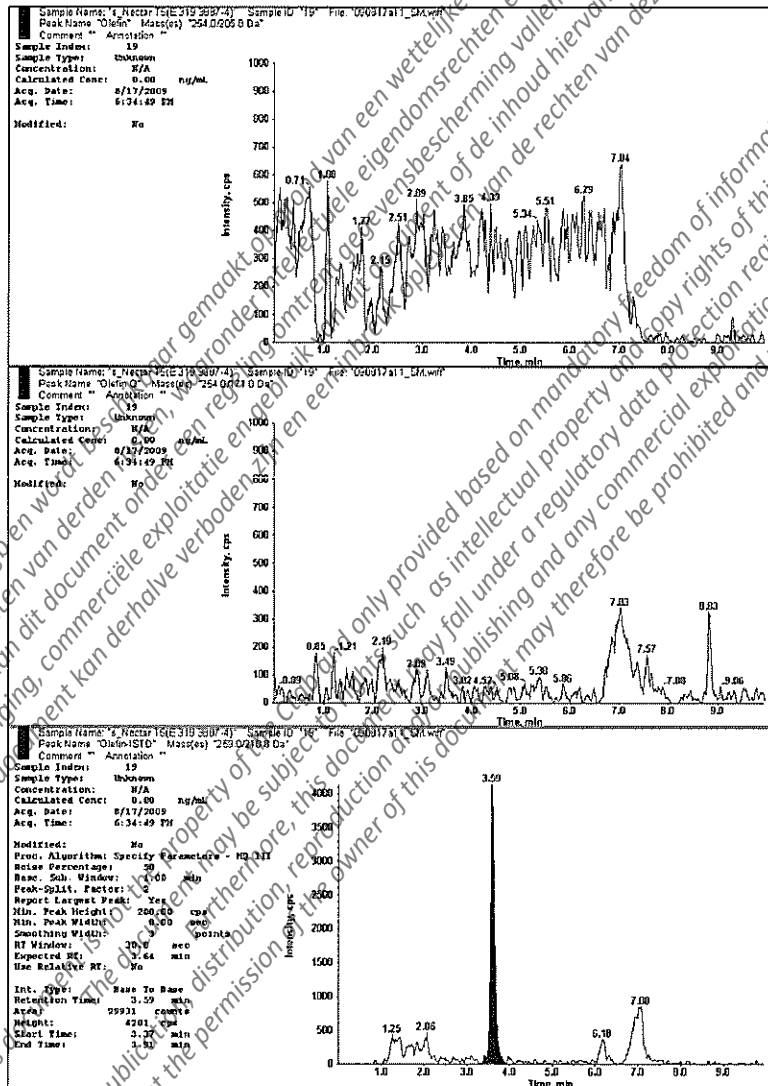
Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1:
Representative Chromatograms (cont'd)

Figure 23 : Treated Sample Nectar T5

Top: NTN33893-olefine, 1st MRM
Middle: NTN33893-olefine, 2nd MRM
Bottom: Internal Standard NTN33893-olefine-¹³C₁₆N



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

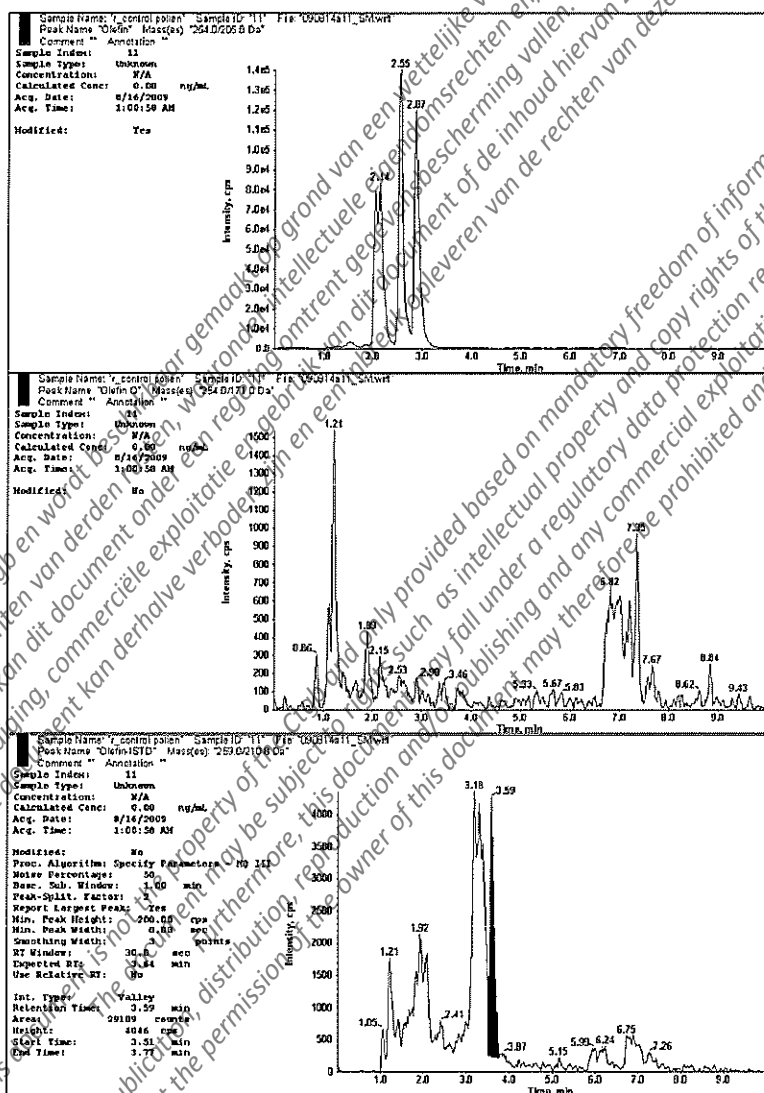
Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1:
Representative Chromatograms (contd)

Figure 24 : Control Sample Pollen

Top: NTN33893-olefine, 1st MRM
Middle: NTN33893-olefine, 2nd MRM
Bottom: Internal Standard NTN33893-olefine-¹³C¹⁵N



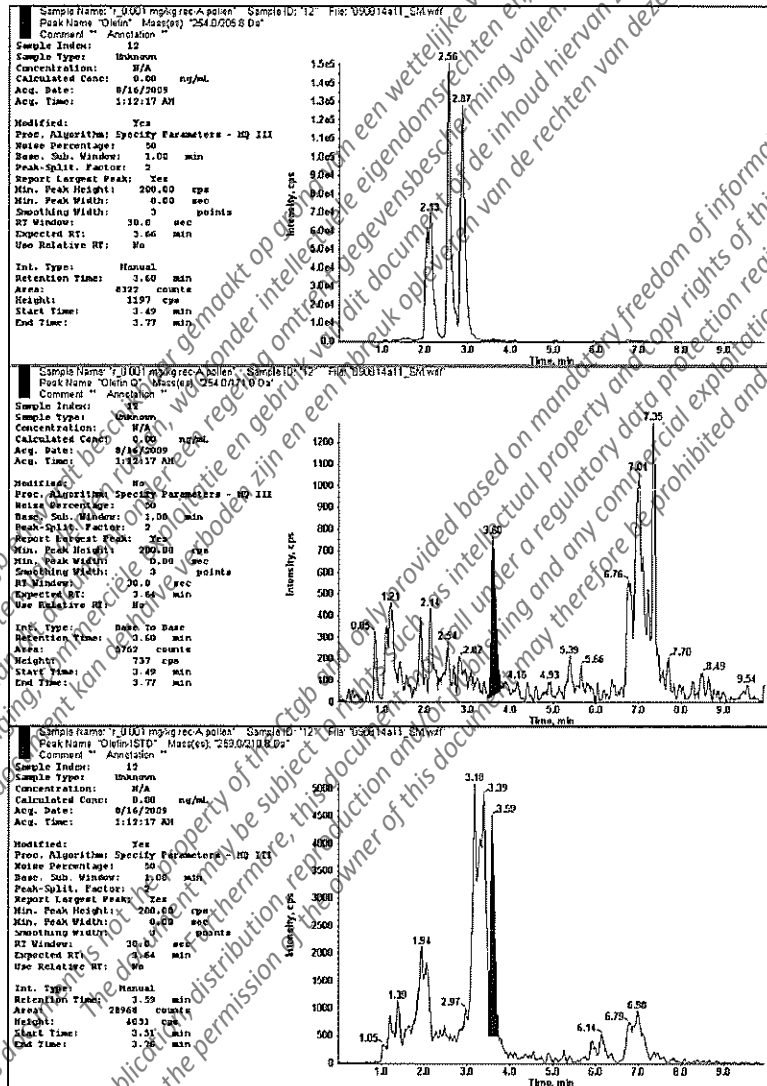
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1: Representative Chromatograms (contd)

Figure 25 : Recovery Sample 0.001 mg/kg NTN33893-olefine in Pollen
Top: NTN33893-olefine, 1st MRM
Middle: NTN33893-olefine, 2nd MRM
Bottom: Internal Standard NTN33893-olefine-¹³C¹⁶N



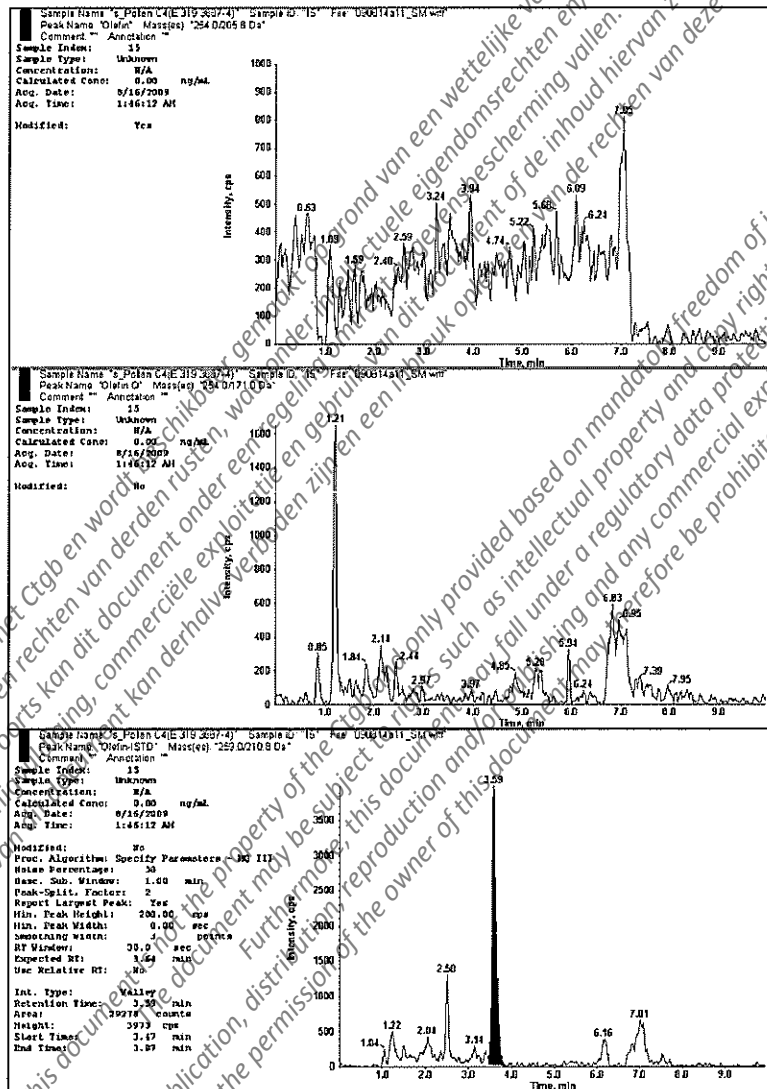
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1:
Representative Chromatograms (contd)

Figure 26 : Control ("Imidacloprid-untreated") Sample Pollen C4
Top: NTN33893-olefine, 1st MRM
Middle: NTN33893-olefine, 2nd MRM
Bottom: Internal Standard NTN33893-olefine-¹³C₁₅N



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

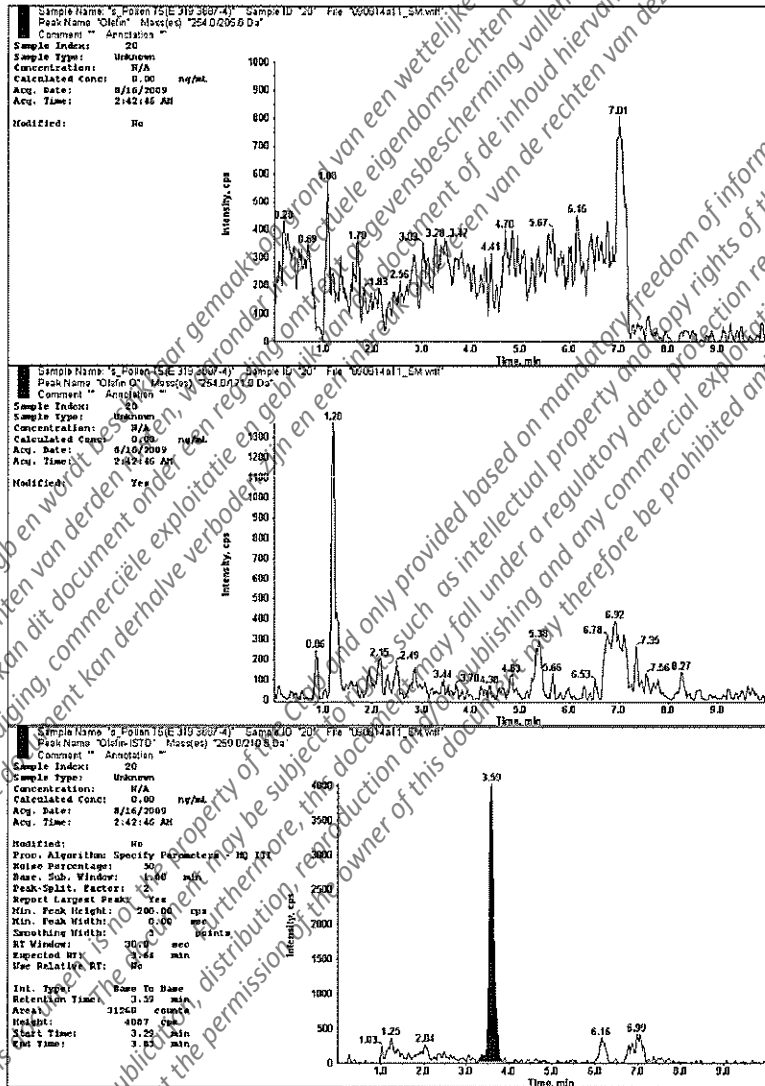
Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 1:
Representative Chromatograms (contd)

Figure 27 : Treated Sample Pollen T5

Top: NTN33893-olefine, 1st MRM
Middle: NTN33893-olefine, 2nd MRM
Bottom: Internal Standard NTN33893-olefine-¹³C₁₆N



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 2:
Detailed Instrument Parameters

<p>Acquisition Method</p> <p>TII & TII ISID & Q 2009-07-29</p> <p>Mass Spec 9.973 min Period 9.973 min +MFI</p> <p>Agilent 1200 ThermoStatted Column Compartment Integrated Valco Valve Agilent 1100 IC Binary Pump (10.0 mins) Equilibrate (0.0 mins) Run (10.0 mins) Agilent 1200 Isocratic Pump (10.0 mins) Equilibrate (0.0 mins) Run (10.0 mins) CTC PAL Autosampler</p>
<p>Acquisition Method Properties</p> <p>Comment:</p> <p>Synchronization Mode: IC Sync Auto Equilibration: Off Acquisition Duration: Unlimited Number of Scans: 519 Periods in File: 1 Acquisition Module: Acquisition Method Software Version: Analyst 1.5</p>

Column: Phenomenex Luna 5µC18 (150 x 4.6 mm)
 Solvent A1: MilliQ-H₂O/acetonitrile (9/1, v/v) + 0.1 mL/L formic acid
 Solvent B1: Acetonitrile + 0.1 mL/L formic acid

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)**Bayer CropScience AG
BCS-D-HS-RA****Study No.: E 319 3387-4****Appendix 2:
Detailed Instrument Parameters (contd)**

Acquisition Method
TII & TII ISID & Q 2009-07-29
Mass Spec 9.973 min
Period 9.973 min
+MS1
Agilent 1200 Thermostatted Column Compartment
Integrated Valco Valve
Agilent 1100 LC Binary Pump (10.0 mins)
 Equilibrate (0.0 mins)
 Run (10.0 mins)
Agilent 1200 Isocratic Pump (10.0 mins)
 Equilibrate (0.0 mins)
 Run (10.0 mins)
CTC PAL Autosampler

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 2:
Detailed Instrument Parameters (contd)

Acquisition Information:

Acquisition Method: TII & TII ISTD & Q 2009-07-29.dam
 Created: Wednesday July 29, 2009 13: 29: 03 RM
 Last Modified: Thursday October 15, 2008 12: 51: 09 RM
 Comment:
 Synchronization Mode: LC Sync
 Auto-Equilibration: Off
 Acquisition Duration: 10min00sec
 Number Of Scans: 518
 Periods In File: 1
 Acquisition Module: Application Method
 Software version: Rev 1.5

Period 1:

Scans in Period: 518
 Relative Start Time: 0.00 msec
 Experiments in Period: 1

Period 1 Experiment 1:

Scan Type: QM (Q1)
 Scheduled MR#: 0
 Polarity: Positive
 Scan Mode: N/A
 Ion Source: Turbo Spray
 Resolution Q1: Unit
 Resolution Q3: Unit
 Intensity Range: 0.00 cps
 Settling Time: 0.000 msec
 MR Pause: 5.000 msec
 MR: 0
 Step Size: 0.00 Da

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	IT
243.94	163.01	50.00	DI	40.00	40.00	TI 435
			CI	17.00	17.00	
			CE	14.00	14.00	
243.94	131.94	50.00	DI	40.00	40.00	TI435-d3
			CI	17.00	17.00	
			CE	10.00	10.00	
252.95	172.01	50.00	DI	40.00	40.00	TI 435-d3
			CI	17.00	17.00	
			CE	14.00	14.00	
206.04	174.97	50.00	DI	50.00	50.00	TI435-T2H
			CI	25.00	25.00	
			CE	10.00	10.00	
206.04	131.94	50.00	DI	50.00	50.00	TI435-T2H Q
			CI	23.00	23.00	
			CE	10.00	10.00	

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 2:
Detailed Instrument Parameters (contd)

Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	Param	Start	Stop	IL
209.02	174.97	50.00	DE	50.00	50.00	T204-62
			CI	21.00	21.00	
			CAF	10.00	10.00	
236.02	131.94	50.00	DE	50.00	50.00	T1935-T204
			CI	21.00	21.00	
			CAF	10.00	10.00	
236.02	113.00	50.00	DE	50.00	50.00	T1935-T204
			CI	25.00	25.00	
			CAF	10.00	10.00	
290.00	133.98	50.00	DE	20.00	20.00	T1202-13C12H
			CI	30.00	30.00	
			CAF	15.00	15.00	
292.02	211.02	50.00	DE	35.00	35.00	T10
			CI	17.00	17.00	
			CAF	18.00	18.00	
294.02	211.02	50.00	DE	20.00	20.00	T10-Q1-C137
			CI	30.00	30.00	
			CAF	15.00	15.00	
254.00	205.00	50.00	DE	41.00	41.00	Olefin
			CI	19.00	19.00	
			CAF	18.00	18.00	
254.00	271.00	50.00	DE	27.00	27.00	Alcitra Q
			CI	19.00	19.00	
			CAF	18.00	18.00	
259.00	210.00	50.00	DE	43.00	43.00	DeLur-ISTD
			CI	19.00	19.00	
			CAF	18.00	18.00	
272.00	191.00	50.00	DE	51.00	51.00	5-hydroxy
			CI	27.00	27.00	
			CAF	18.00	18.00	
272.00	146.16	50.00	DE	51.00	51.00	5-hydroxy Q
			CI	43.00	43.00	
			CAF	14.00	14.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	Param	Start	Stop	IL

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

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Appendix 2:
Detailed Instrument Parameters (contd)

277.0C	196.0C	50.0C	DF	51.0C	51.00	5-hydroxy-ISTD		
			CE	27.0C	27.00			
			CF	18.0C	18.00			
Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	Ratio	Start	Stop	IL		
256.0C	175.0C	50.0C	DF	51.00	51.00	Imidacloprid		
			CE	27.00	27.00			
			CF	16.00	16.00			
Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	Ratio	Start	Stop	IL		
256.0C	209.8C	50.0C	DF	51.00	51.00	Imidacloprid		
			CE	23.00	23.00			
			CF	14.00	14.00			
Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	Ratio	Start	Stop	IL		
261.0C	179.0C	50.0C	DF	26.00	20.00	[d5-13C] Imidacloprid		
			CE	30.00	30.00			
			CF	15.00	15.00			
Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	Ratio	Start	Stop	IL		
254.0C	235.8C	50.0C	DF	41.00	41.00	clafin 18		
			CE	14.00	13.00			
			CF	15.00	15.00			
Parameter Table (Period 1 Experiment)								
CR:	20.00							
GS1:	09.00							
GS2:	09.00							
IS:	4500.00							
TEM:	650.00							
lhc:	00							
CPD:	6.00							
EP:	18.00							
Agilent Column Oven Properties								
Left Temperature (°C):				50.00				
Right Temperature (°C):				50.00				
Temperature tolerance +/- (°C):				1.00				
Start Acquisition Tolerance +/- (°C):				0.50				
Temp Table (Not Used)								
Column Switching Valve	Installed							
Position for first sample in the batch:	Left							
Use same position for all samples in the batch:								
Valve Valve	Diverter							
	Total Time (min)							
1	2.0	A						
2	7.0	B						
Agilent IC Pump Method Properties								
Pump Model:	Agilent 1100 IC Binary Pump							
Minimum Pressure (psi):	0.0							
Maximum Pressure (psi):	580.0							
Dead Volume (µl):	40.0							
Maximum Flow Ramp (µl/min):	100.0							
Maximum Pressure Ramp (psi/sec):	290.0							
Step Table:								
Step	Total Time (min)	Flow Rate (µl/min)	A (%)	B (%)	TEM1	TEM2	TEM3	TEM4
0	0.00	1250	88.0	12.0	open	open	open	open
1	50.50	1250	88.0	12.0	open	open	open	open
2	56.00	1250	10.0	90.0	open	open	open	open
3	8.00	1250	10.0	90.0	open	open	open	open

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
BCS-D-HS-RA

Study No.: E 319 3387-4

Appendix 2: Detailed Instrument Parameters (contd)

4	8.10	1250	68.0	12.0	open	open	open	open
5	10.00	1250	68.0	12.0	open	open	open	open

Left Compressibility: 50.0
Right Compressibility: 115.0
Left Dead Volume (µl): 40.0
Right Dead Volume (µl): 40.0
Left Stroke Volume (µl): -1.0
Right Stroke Volume (µl): -1.0
Left Solvent: A1
Right Solvent: B1

Agilent IC Pump Method Properties
Pump Model: Agilent 1200 Isocratic Pump
Minimum Pressure (psi): 0.0
Maximum Pressure (psi): 5901.0
Compressibility: 100.0
Dead Volume (µl): 40.0
Stroke Volume (µl): -1.0
Maximum Flow Ramp (ml/min): 100.0
Maximum Pressure Ramp (psi/sec): 250.0

Step Table:

Step	Total Time (min)	Flow Rate (µl/min)	TS#1	TS#2	TS#3	TS#4
0	0.00	1250	open	open	open	open
1	10.00	1250	open	open	open	open

CIC PAL Autosampler Method Properties
Loop Volume1 (µl): 100
Loop Volume2 (µl): 100
Injection Volume (µl): 100/100
Method Description:

Syringe: 100µl
01 Analyze LC-Int
Air Volume (µl) 0
Pre Clean with Solvent 1 () 1
Pre Clean with Solvent 2 () 1
Pre Clean with Sample () 0
Filling Speed (µl/s) 10
Filling Strokes () 0
Inject to (V1/V2) 1/2
Injection Speed (µl/s) 100
Pre Inject Delay (ms) 500
Post Inject Delay (ms) 500
Post Clean with Solvent 1 () 1
Post Clean with Solvent 2 () 1
Valve Clean with Solvent 1 () 1
Valve Clean with Solvent 2 () 1
Replicate Count () 1
Analysis Time (s) () 0

Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

Bayer CropScience AG
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Study No.: E 319 3387-4

Appendix 3:
Representative Linearity Plots

Figure 28: Linearity Imidacloprid (Range of Concentration: 0.1 – 10 ng/mL corresponding to 0.0001 – 0.01 mg/kg)

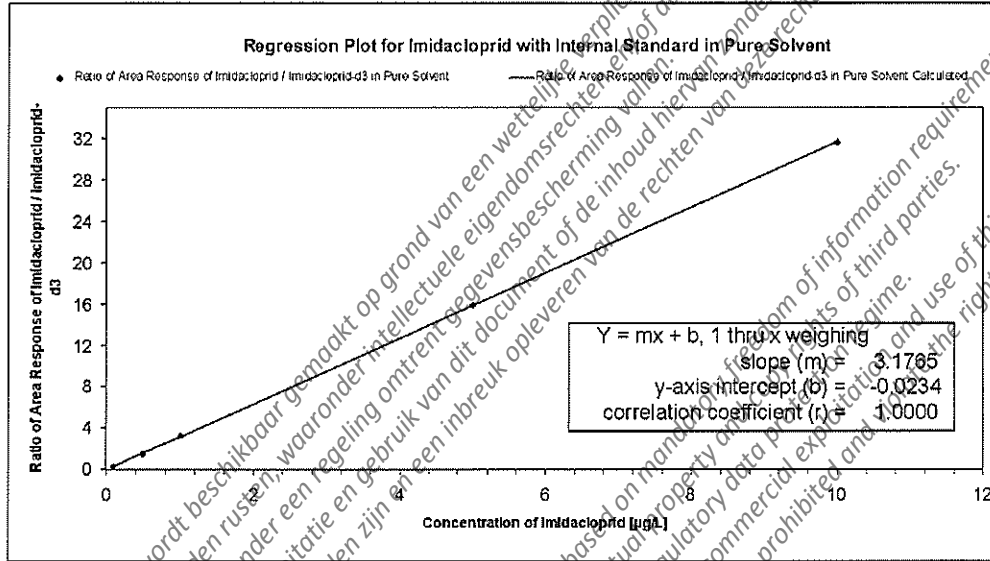
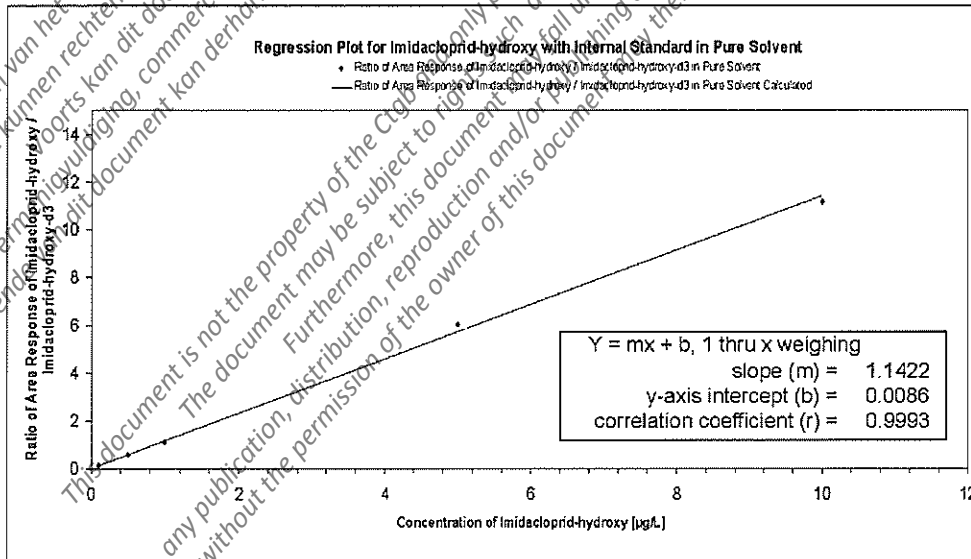


Figure 29: Linearity Imidacloprid-mono-hydroxy (Range of Concentration: 0.1 – 10 ng/mL corresponding to 0.0001 – 0.01 mg/kg)



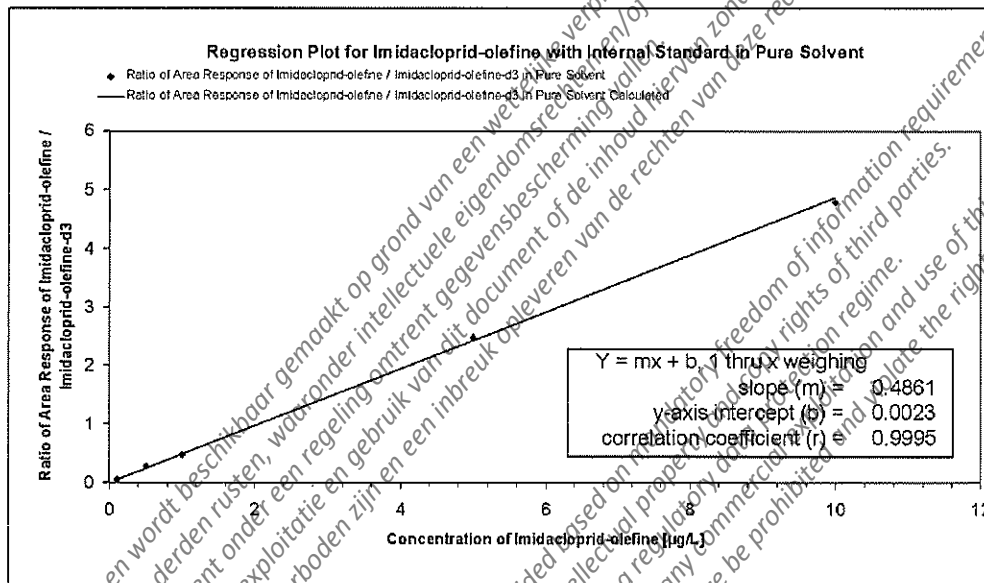
Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

**Bayer CropScience AG
BCS-D-HS-RA**

Study No.: E 319 3387-4

**Appendix 3:
Representative Linearity Plots (contd)**

Figure 30: Linearity Imidacloprid-olefine (Range of Concentration: 0.1 – 10 ng/mL corresponding to 0.0001 – 0.01 mg/kg)



Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

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Study No.: E 319 3387-4



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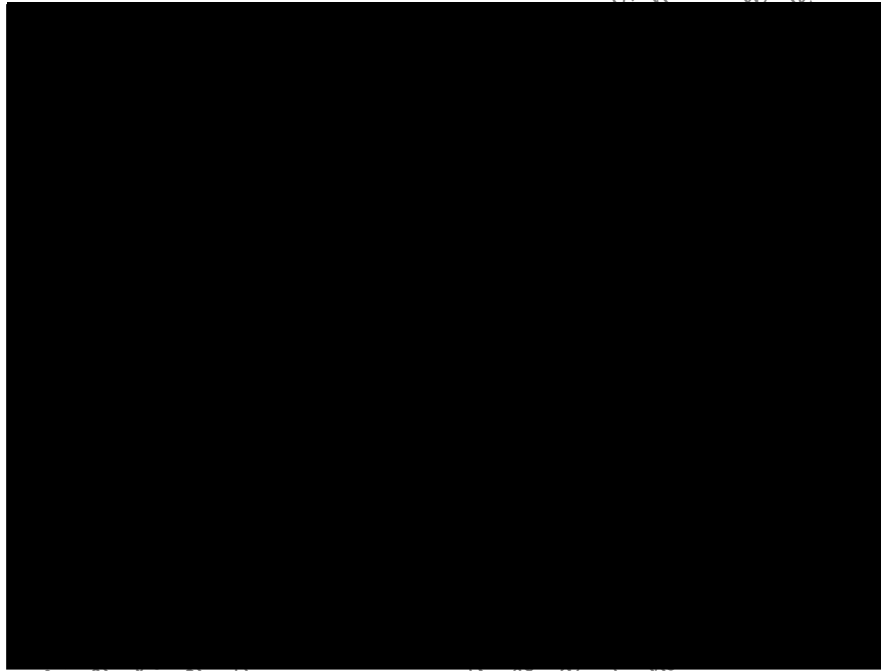
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Appendix 6 Analytical Phase Report Nectar and Pollen Analysis (continued)

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Study No.: E 319 3387-4

Appendix 4: GLP Certificate (contd)



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