

**STUDY TITLE**

**Residue Levels of Imidacloprid and Imidacloprid Metabolites in Sunflower Pollen,  
Sunflower Honey and Bees from Gaucho Treated Sunflowers in the Field**

Test Location: farmland "Ahrweiler Mayen"

**Author**

[Redacted]

**Test Facility**

Bayer CropScience AG  
Development – Residues, Operator and Consumer Safety  
BCS-D-ROCS, Building 6610  
40789 Monheim, Germany

**Study Completion Date**

January 21, 2003

**Study Number**

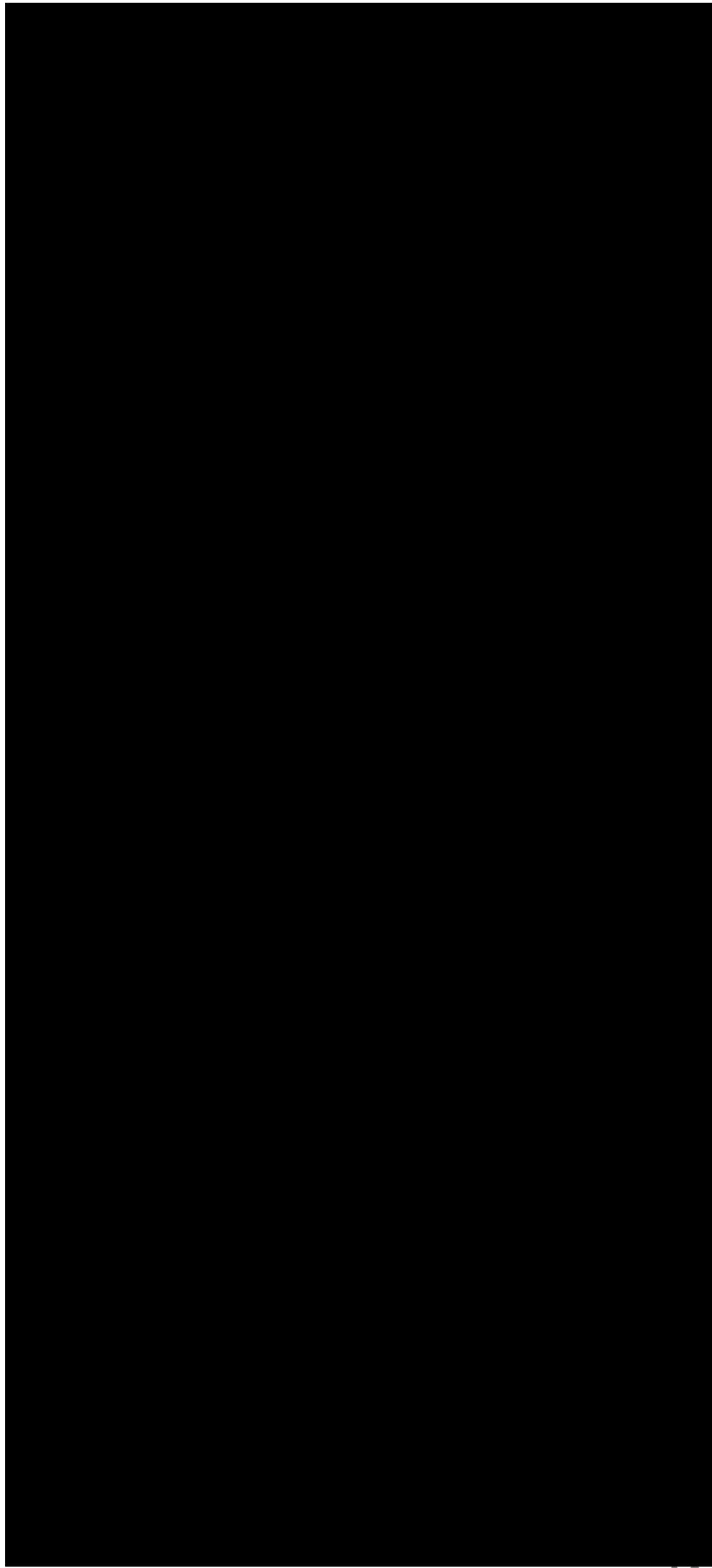
E 370 1742-4



MR-710/99 / MO-03-000765

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CERTIFICATION OF AUTHENTICITY



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2003-01-21  
Date

Head of ROCS

2003-01-21  
Date


Study Director and Head of ETX

2003-01-21  
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GOOD LABORATORY PRACTICE STATEMENT

Study Number E 370 1742-4

Test Substance Gaucho (a.i. Imidacloprid)

Study Director [Redacted]

Test Facility Bayer CropScience AG, BCS-D-ETX (formerly Bayer AG, PF-E/OE)

Study Title Residue Levels of Imidacloprid and Imidacloprid Metabolites in Sunflower Pollen, Sunflower Honey and Bees from Gaucho Treated Sunflowers in the Field

These studies were conducted in compliance with the Principles of Good Laboratory Practice (Chemikaliengesetz, dated July 25, 1994, current version of Annex 1; OECD Principles of Good Laboratory Practice (GLP), dated November 26, 1997 [C(97) 186/Final]).

The test facility was inspected and certified as working in compliance with the principles of Good Laboratory Practice by the competent authority ("Aktenzeichen" IV C4 - 31.11.60.03, March 4, 1999).

Responsible Analyst Bayer CropScience AG

[Redacted Signature]

2003-01-21

Date: yyyy-mm-dd

Head of ETX

[Redacted Signature]

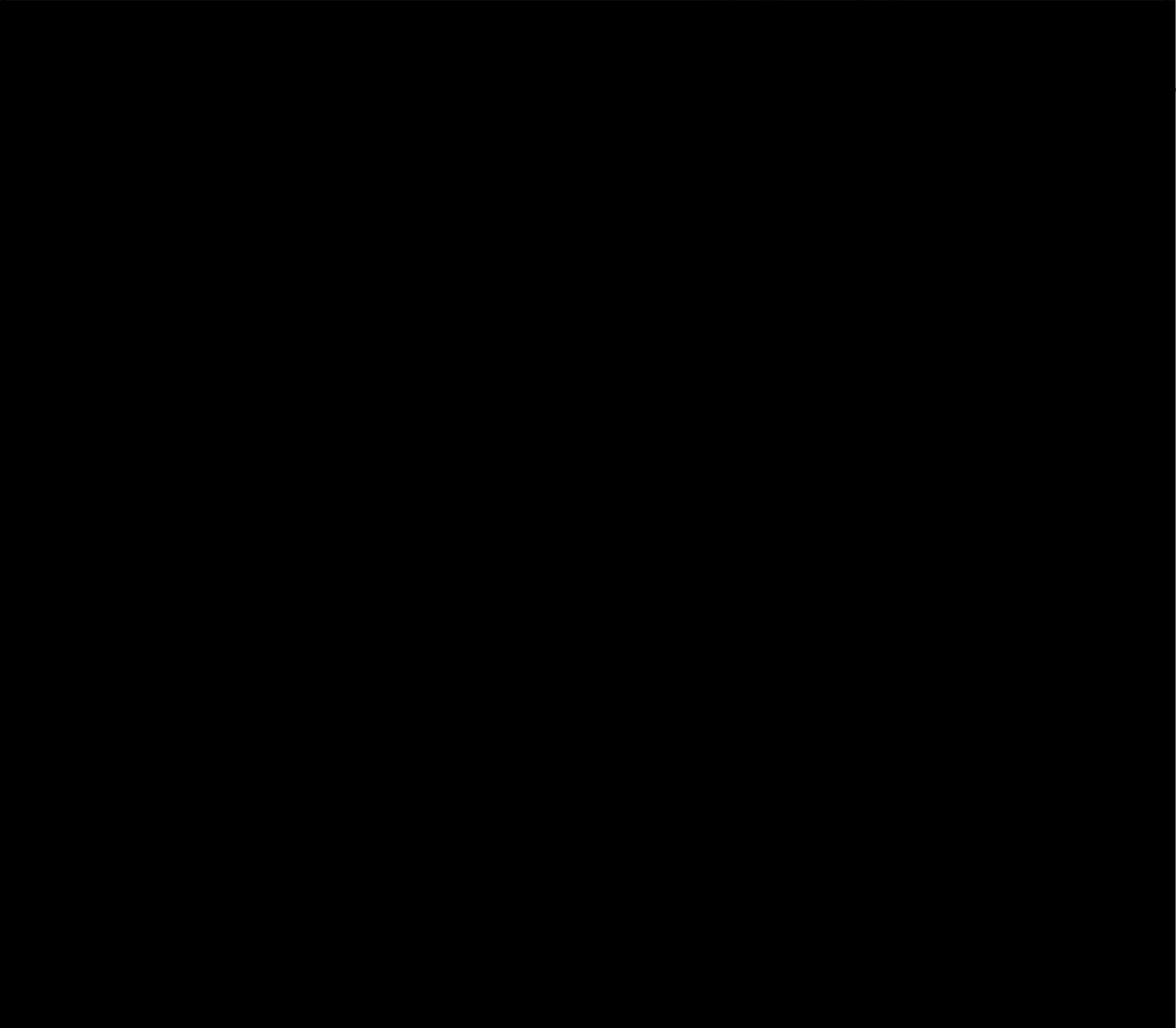
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QA STATEMENT

<b>BCS-D-AS-GLP/QAU</b>
<b>Quality Assurance Statement</b>



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## 1 INTRODUCTION

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

## 2 TIME SCHEDULE

The experimental work was performed during the following time period:

Signature of study protocol: October 01, 1999  
 Start of experimental phase: October 04, 1999  
 End of experimental phase: December 03, 1999  
 Completion of report: January 21, 2003

## 3 RESULTS FOR SUNFLOWER HONEY, POLLEN AND BEE SAMPLES

### 3.1 Fresh Honey Samples Wörrstadt:

Sample Name	Sample description	Hydroxy- Imidacloprid [mg/kg]	Olefin- Imidacloprid [mg/kg]	Imidacloprid [mg/kg]
Honig 50 K Wörrstadt	Honey of Bee swarm No. 50 Control, Wörrstadt	n.d.	n.d.	n.d.
Honig 98 K Wörrstadt	Honey of Bee swarm No. 98, Control, Wörrstadt	n.d.	n.d.	n.d.
Honig 122 K Wörrstadt	Honey of Bee swarm No. 122 Control, Wörrstadt	n.d.	n.d.	n.d.
Honig 212 K Wörrstadt	Honey of Bee swarm No. 212, Control, Wörrstadt	n.d.	n.d.	n.d.

Limit of quantitation: 0.005 mg/kg for imidacloprid and hydroxy-metabolite, 0.01 mg/kg for the olefin-metabolite, < 0.005 and < 0.010 = Residues below the limit of quantitation  
 Limit of detection: 0.0015 mg/kg for imidacloprid and hydroxy-metabolite, 0.003 mg/kg for the olefin-metabolite, n.d.: Residues below the limit of detection

### 3.2 Fresh Honey Samples Rodderhöfe:

Sample Name	Sample description	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]	Imidacloprid [mg/kg]
Honig 50 G Rodderhöfe	Honey from Bee swarm No. 50, Gaucho, Rodderhöfe	n.d.	n.d.	n.d.
Honig 65 G Rodderhöfe	Honey of Bee swarm No. 65, Gaucho, Rodderhöfe	n.d.	n.d.	n.d.
Honig 98 G Rodderhöfe	Honey of Bee swarm No. 98, Gaucho, Rodderhöfe	n.d.	n.d.	n.d.
Honig 173 G Rodderhöfe	Honey of Bee swarm No. 173, Gaucho, Rodderhöfe	n.d.	n.d.	n.d.
Honig 229 G Rodderhöfe	Honey of Bee swarm No. 229, Gaucho, Rodderhöfe	n.d.	n.d.	n.d.

Limit of quantitation: 0.005 mg/kg for imidacloprid and hydroxy-metabolite, 0.01 mg/kg for the olefin-metabolite, < 0.005 and < 0.010 = Residues below the limit of quantitation  
 Limit of detection: 0.0015 mg/kg for imidacloprid and hydroxy-metabolite, 0.003 mg/kg for the olefin-metabolite, n.d.: Residues below the limit of detection

### 3.3 Fresh Honey Samples Groß-Winterheim:

Sample Name	Sample description	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]	Imidacloprid [mg/kg]
Honig 23 G Groß-Winterheim	Honey of Bee swarm No. 23, Gaucho, Groß-Winterheim	n.d.	n.d.	n.d.
Honig 136 G Groß-Winterheim	Honey of Bee swarm No. 136, Gaucho, Groß-Winterheim	n.d.	n.d.	n.d.
Honig 173 G Groß-Winterheim	Honey of Bee swarm No. 173, Gaucho, Groß-Winterheim	n.d.	n.d.	n.d.
Honig 206 G Groß-Winterheim	Honey of Bee swarm No. 206, Gaucho, Groß-Winterheim	n.d.	n.d.	n.d.

Limit of quantitation: 0.005 mg/kg for imidacloprid and hydroxy-metabolite, 0.01 mg/kg for the olefin-metabolite, < 0.005 and < 0.010 = Residues below the limit of quantitation  
 Limit of detection: 0.0015 mg/kg for imidacloprid and hydroxy-metabolite, 0.003 mg/kg for the olefin-metabolite, n.d.: Residues below the limit of detection

## 3.4 Pollen Samples from the Pollen Trap:

Sample Name	Swarm No.	Colour	Test-variant	Test Location	Date	Hydroxy-IMI [mg/kg]	Olefin-IMI [mg/kg]	Imidacloprid [mg/kg]
Pollen 17424K128M001	128	-	Control	Wörrstadt	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424G234R002	234	red	Gaucho	-	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424G234O003	234	orange	Gaucho	-	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424G234G004	234	yellow	Gaucho	-	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424G234B005	234	blue	Gaucho	-	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424G222O006	222	orange	Gaucho	-	7.-9.07.99	n.d.	n.d.	< LOQ
Pollen 17424G222LH007	222	light lilac	Gaucho	-	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424G222R008	222	red	Gaucho	-	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424G222G009	222	yellow	Gaucho	-	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424G222RD010	222	dark red	Gaucho	-	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424G222RP011	222	-	Gaucho	-	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424KO012	-	orange	Control	Wörrstadt	11.07.99	n.d.	n.d.	n.d.
Pollen 17424KG014	-	yellow	Control	Wörrstadt	11.07.99	n.d.	n.d.	n.d.
Pollen 17424KL015	-	lilac	Control	Wörrstadt	11.07.99	n.d.	n.d.	n.d.
Pollen 17424G222234R016	222 234	red	Gaucho	-	11.07.99	n.d.	n.d.	n.d.
Pollen 17424G222234G017	222 234	yellow	Gaucho	-	11.07.99	n.d.	n.d.	n.d.
Pollen 17424G222234OL018	222 234	olive	Gaucho	-	11.07.99	n.d.	n.d.	n.d.
Pollen 17424G222234LH019	222 234	light lilac	Gaucho	-	11.07.99	n.d.	n.d.	n.d.
Pollen 17424G222234O020	222 234	orange	Gaucho	-	11.07.99	n.d.	n.d.	n.d.
Pollen 17424G222234L021	222 234	lilac	Gaucho	-	11.07.99	n.d.	n.d.	n.d.
Pollen 17424K128G022	128	yellow	Control	Wörrstadt	13.07.99	n.d.	n.d.	n.d.



## 3.4 Pollen Samples from the Pollen Trap (cont.):

Sample Name	Swarm No.	Colour	Test-variant	Test Location	Date	Hydroxy-IMI [mg/kg]	Olefin-IMI [mg/kg]	Imidacloprid [mg/kg]
Pollen 17424K128L023	128	lilac	Control	Wörrstadt	13.07.99	n.d.	n.d.	n.d.
Pollen 17424K200L026	200	lilac	Control	Wörrstadt	13.07.99	n.d.	n.d.	n.d.
Pollen 17424K200O027	200	orange	Control	Wörrstadt	13.07.99	n.d.	n.d.	n.d.
Pollen 17424K200R028	200	red	Control	Wörrstadt	13.07.99	n.d.	n.d.	n.d.
Pollen 17424K200G029	200	yellow	Control	Wörrstadt	13.07.99	n.d.	n.d.	n.d.
Pollen 17424G234OL030	234	olive	Gaucho	-	13.07.99	n.d.	n.d.	n.d.
Pollen 17424G234G031	234	yellow	Gaucho	-	13.07.99	n.d.	n.d.	n.d.
Pollen 17424G234R032	234	red	Gaucho	-	13.07.99	n.d.	n.d.	n.d.
Pollen 17424G234LH033	234	light lilac	Gaucho	-	13.07.99	n.d.	n.d.	n.d.
Pollen 17424G222L035	222	lilac	Gaucho	-	13.07.99	n.d.	n.d.	n.d.
Pollen 17424G222LH036	222	light lilac	Gaucho	-	13.07.99	n.d.	n.d.	n.d.
Pollen 17424G222O037	222	orange	Gaucho	-	13.07.99	n.d.	n.d.	< LOQ
Pollen 17424G222OL038	222	olive	Gaucho	-	13.07.99	n.d.	n.d.	n.d.
Pollen 17424G222G039	222	yellow	Gaucho	-	13.07.99	n.d.	n.d.	n.d.
Pollen 17424G222R040	222	red	Gaucho	-	13.07.99	n.d.	n.d.	n.d.
Pollen 17424KOL042	-	olive	Control	Wörrstadt	19.07.99	n.d.	n.d.	n.d.
Pollen 17424KLH043	-	light lilac	Control	Wörrstadt	19.07.99	n.d.	n.d.	n.d.
Pollen 17424KL044	-	lilac	Control	Wörrstadt	19.07.99	n.d.	n.d.	n.d.
Pollen 17424GO045	-	orange	Gaucho	Groß-Winterheim	19.07.99	n.d.	n.d.	n.d.
Pollen 17424GOL046	-	olive	Gaucho	Groß-Winterheim	19.07.99	n.d.	n.d.	n.d.

## 3.4 Pollen Samples from the Pollen Trap (cont.):

Sample Name	Swarm No.	Colour	Test Variant	Test Location	Date	Hydroxy-IMI [mg/kg]	Olefin-IMI [mg/kg]	Imidacloprid [mg/kg]
Pollen 17424GLH047	-	light lilac	Gaucho	Groß-Winterheim	19.07.99	n.d.	n.d.	n.d.
Pollen 17424GL048	-	lilac	Gaucho	Groß-Winterheim	19.07.99	n.d.	n.d.	n.d.
Pollen 17424K200G050	200	yellow	Control	Wörrstadt	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424K200HL051	200	light lilac	Control	Wörrstadt	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424K200R052	200	red	Control	Wörrstadt	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424K200/2G053	200/2	yellow	Control	Wörrstadt/2	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424K200/2HL054	200/2	light lilac	Control	Wörrstadt/2	7.-9.07.99	n.d.	n.d.	n.d.
Pollen 17424K200/2R055	200/2	red	Control	Wörrstadt/2	7.-9.07.99	n.d.	n.d.	n.d.

Limit of quantitation: 0.005 mg/kg for imidacloprid and hydroxy-metabolite, 0.01 mg/kg for the olefin-metabolite, < 0.005 and < 0.010 = Residues below the limit of quantitation.  
 Limit of detection: 0.0015 mg/kg for imidacloprid and hydroxy-metabolite, 0.003 mg/kg for the olefin-metabolite, n.d.: Residues below the limit of detection

## 3.5 Fresh Sunflower Florets and Pollen Samples:

Sample Name	Test Variant	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]	Imidacloprid [mg/kg]
Sunflower Florets	Gaucho	n.d.	n.d.	< LOQ
Sunflower Pollen	Gaucho	n.d.	n.d.	< LOQ

Limit of quantitation: 0.005 mg/kg for imidacloprid and hydroxy-metabolite, 0.01 mg/kg for the olefin-metabolite, < 0.005 and < 0.010 = Residues below the limit of quantitation  
 Limit of detection: 0.0015 mg/kg for imidacloprid and hydroxy-metabolite, 0.003 mg/kg for the olefin-metabolite, n.d.: Residues below the limit of detection

### 3.6 Intact Bees:

Sample Name	Test Location	Date	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]	Imidacloprid [mg/kg]
Bees Sample 1	Groß-Winterheim	19.07.99	n.d.	n.d.	n.d.
Bees Sample 2	Groß-Winterheim	19.07.99	n.d.	n.d.	n.d.
Bees	Rodderhöfe	01.07.99	n.d.	n.d.	n.d.

Limit of quantitation: 0.005 mg/kg for imidacloprid and hydroxy-metabolite, 0.01 mg/kg for the olefin-metabolite, < 0.005 and <0.010 = Residues below the limit of quantitation  
 Limit of detection: 0.0015 mg/kg for imidacloprid and hydroxy-metabolite, 0.003 mg/kg for the olefin-metabolite, n.d.: Residues below the limit of detection

### 3.7 Content of Honeybulbs:

Sample Name	Test Location	Date	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]	Imidacloprid [mg/kg]
Nectare of Bees Sample 1	Groß-Winterheim	19.07.99	n.d.	n.d.	< LOQ
Nectare of Bees Sample 2	Groß-Winterheim	19.07.99	n.d.	n.d.	< LOQ
Nectare of Bees	Rodderhöfe	01.07.99	n.d.	n.d.	n.d.

Limit of quantitation: 0.005 mg/kg for imidacloprid and hydroxy-metabolite, 0.01 mg/kg for the olefin-metabolite, < 0.005 and <0.010 = Residues below the limit of quantitation  
 Limit of detection: 0.0015 mg/kg for imidacloprid and hydroxy-metabolite, 0.003 mg/kg for the olefin-metabolite, n.d.: Residues below the limit of detection

### 3.8 Dissected Honey Bees:

Sample Name	Test Location	Date	Hydroxy-Imidacloprid [mg/kg]	Olefin-Imidacloprid [mg/kg]	Imidacloprid [mg/kg]
Bees Sample 1	Groß-Winterheim	19.07.99	n.d.	n.d.	n.d.
Bees Sample 2	Groß-Winterheim	19.07.99	n.d.	n.d.	n.d.
Bees	Rodderhöfe	01.07.99	n.d.	n.d.	n.d.

Limit of quantitation: 0.005 mg/kg for imidacloprid and hydroxy-metabolite, 0.01 mg/kg for the olefin-metabolite, < 0.005 and <0.010 = Residues below the limit of quantitation  
 Limit of detection: 0.0015 mg/kg for imidacloprid and hydroxy-metabolite, 0.003 mg/kg for the olefin-metabolite, n.d.: Residues below the limit of detection

## 4 RECOVERIES

The analytical method for imidacloprid and metabolites was validated by performing recovery experiments during the analyses of the samples. Control samples were fortified with imidacloprid and the hydroxy metabolite at 0.005 mg/kg and at 0.01 mg/kg for the olefin metabolite (LOQ). Recovery data for the parent compound and the metabolites are given in the Tables below.

### Recovery Data for Imidacloprid

FL: Fortification Level, RSD: Relative Standard Deviation, LOQ: Practical Limit of Quantitation

Sample Material	Analyte	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Honey	Imidacloprid	0.005	101, 95	98	3.9	0.005
Pollen			96, 100, 99, 100, 90, 96	97		
Bees			93	93		
<b>Overall Recovery Imidacloprid, n=9</b>				<b>97</b>	<b>3.8</b>	

Final determination as : imidacloprid, Residues calculated as : imidacloprid

### Recovery Data for Hydroxy Metabolite

FL: Fortification Level, RSD: Relative Standard Deviation, LOQ: Practical Limit of Quantitation

Sample Material	Analyte	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Honey	Hydroxy Metabolite	0.005	97, 94	96	6.9	0.005
Pollen			98, 99, 96, 94, 82, 99	95		
Bees			81	81		
<b>Overall Recovery Hydroxy Metabolite, n=9</b>				<b>93</b>	<b>7.5</b>	

Final determination as : hydroxy metabolite, Residues calculated as : hydroxy metabolite

### Recovery Data for Olefin Metabolite

FL: Fortification Level, RSD: Relative Standard Deviation, LOQ: Practical Limit of Quantitation

Sample Material	Analyte	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Honey	Olefin Metabolite	0.01	97, 94	96	5.3	0.01
Pollen			98, 102, 95, 91, 104, 104	99		
Bees			97	97		
<b>Overall Recovery Olefin Metabolite, n=9</b>				<b>98</b>	<b>4.6</b>	

Final determination as : olefin metabolite, Residues calculated as : olefin metabolite

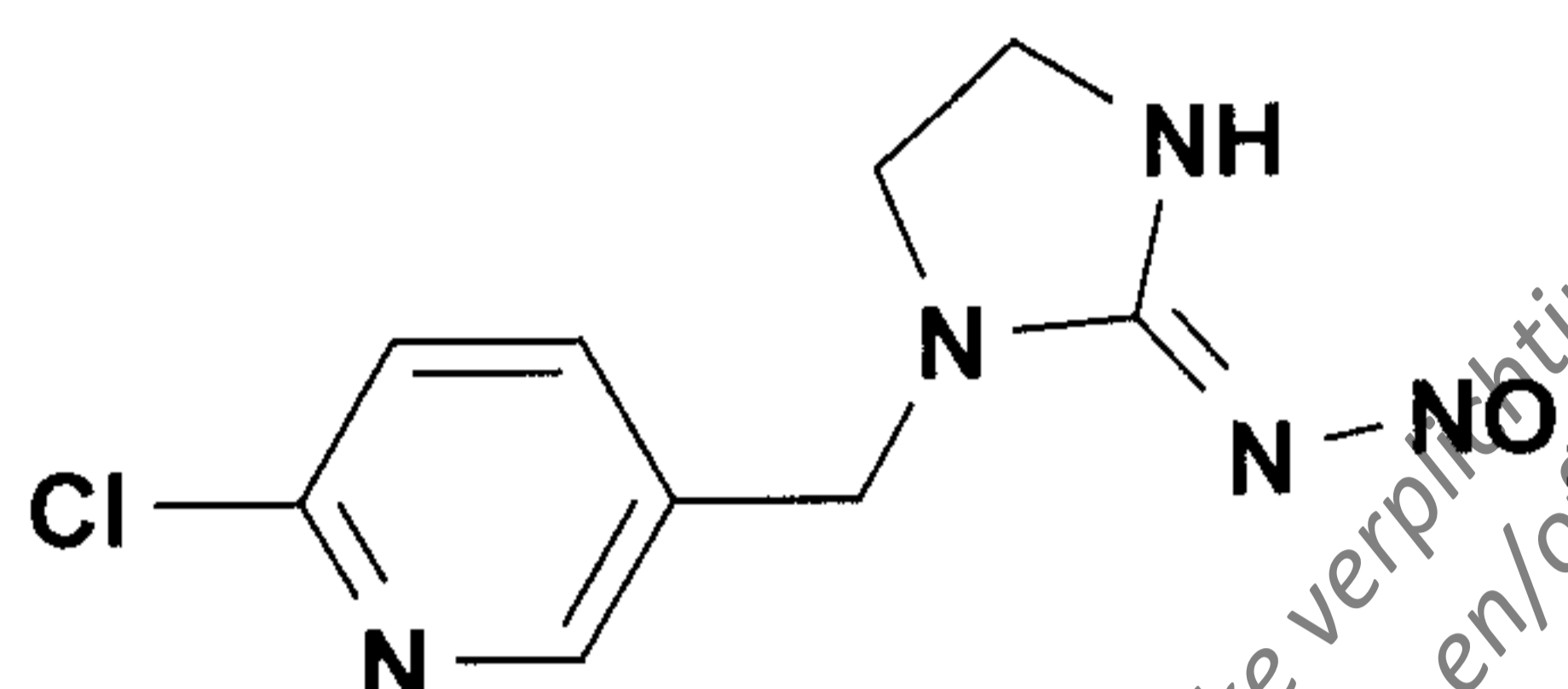
The single recovery values range from 81 to 104% and the overall recoveries from 93 to 97% with relative standard deviations from 3.8 to 7.5% (n=9) for all compounds. All results of the recoveries are in accordance with the general requirements for residue analytical methods.

## 5 EXPERIMENTAL

## 5.1 Reference Substances

**Imidacloprid**

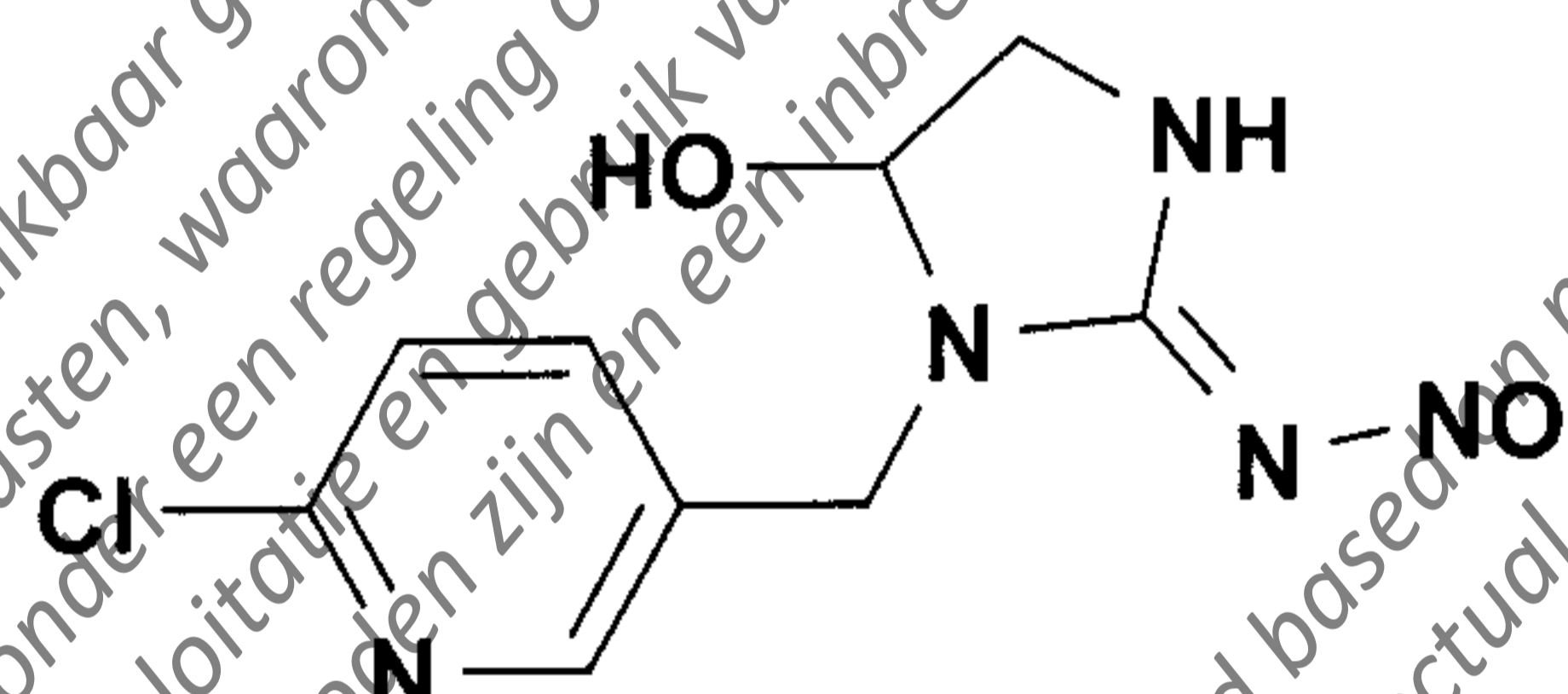
Structural formula:



Empirical formula:  $C_9H_{10}ClN_5O_2$   
 Molecular weight: 255.7 g/mol  
 Certificate of analysis: M00680, 1998-03-30  
 Certified assay: 99.4 %  
 Expiry date: March 2000

**Hydroxy-Imidacloprid (WAK 4103)**

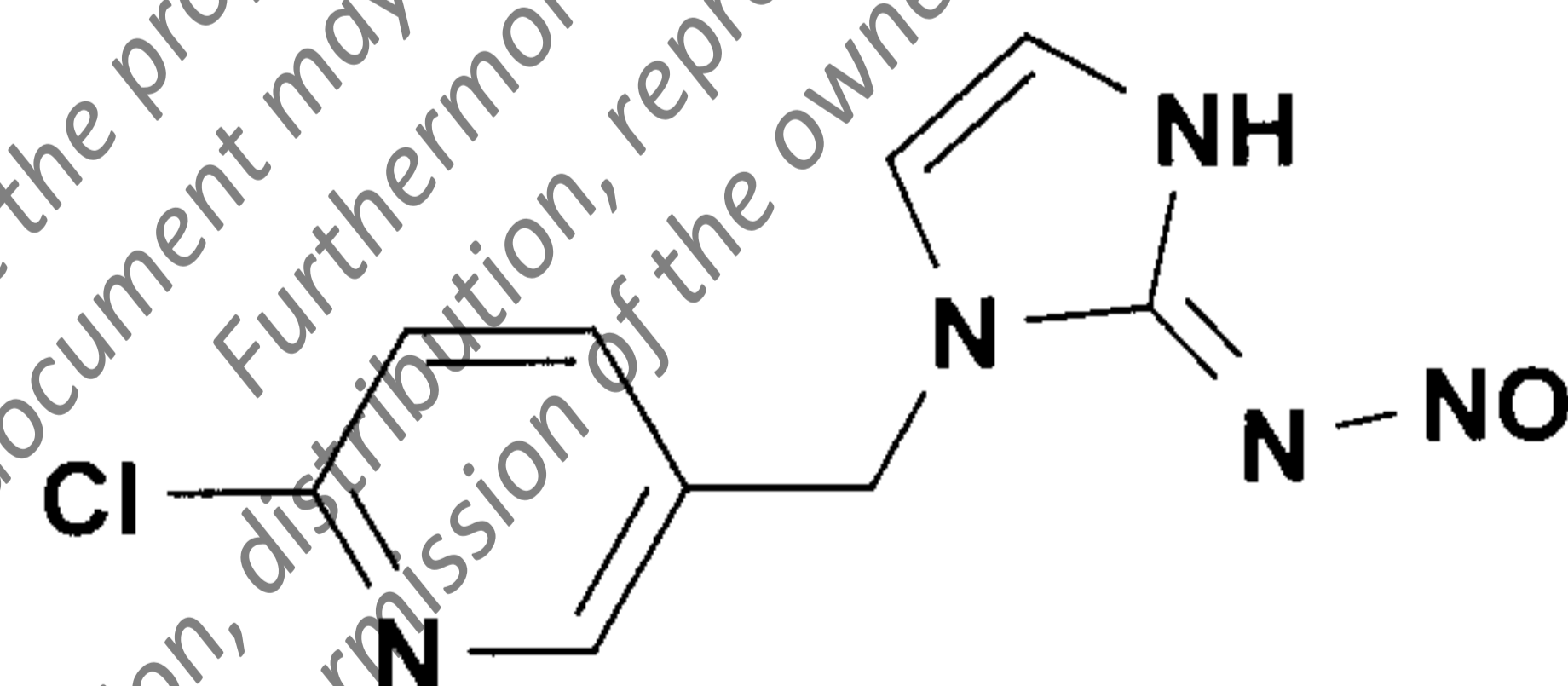
Structural formula:



Empirical formula:  $C_9H_{10}ClN_5O_4$   
 Molecular weight: 271.7 g/mol  
 Certificate of analysis: 930323ELB03, 1995-06-075  
 Certified assay: 99.4 %  
 Expiry date: June 2000

**Olefin-Imidacloprid (NTN 35884)**

Structural formula:



Empirical formula:  $C_9H_8ClN_5O_2$   
 Molecular weight: 253.6 g/mol  
 Certificate of analysis: M00804, 1998-07-22  
 Certified assay: 98 %  
 Expiry date: June 2000

## 5.2 Residue Analytical Methodology

### 5.2.1 Extraction and Sample Clean-up

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

### 5.2.2 ChemElut® Column Clean-up

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

### 5.2.3 Silica Gel Column Clean-up

1. Dissolve the residues from 5.2.2 step 4 in 2 mL of toluene/ethyl acetate (85/15, v/v).
2. Apply the organic solution from step 1 onto a 0.5 g (3 mL) silica gel (SiOH) column (e.g. Varian).
3. Allow the solution to pass through the column at a flow rate of 1 mL/min.
4. Rinse the 250-mL brown glass round-bottomed flask with 10 mL of toluene/ethyl acetate (70/30, v/v) and apply the solution onto the column, too.
5. Elute the residues with 5 mL of acetonitrile at a flow rate of 1 mL/min. Collect the eluate in a 25-mL brown glass pear-shaped flask.
6. Evaporate the eluate from step 5 to dryness using a vacuum rotary evaporator and a max. bath temperature of 40° C. Dissolve the residues in e.g. 1.00 mL of acetonitrile/water (2/8, v/v) and determine the residues by HPLC-MS/MS.

#### NOTE

1. The volumes to be used for flushing the column with toluene/ethyl acetate and for elution with acetonitrile must be newly determined for each batch of SiOH-column!
2. The flow rate should not be too high, since otherwise losses of the residues may occur with recoveries below 70 % and a less effective clean-up.
3. The hydroxy-metabolite may be converted to the olefin-metabolite (especially under acidic conditions).
4. The olefin-metabolite is degraded by light (ca. 50 % in one day at natural daylight). Therefore, all solutions containing the olefin-metabolite must be protected from light and stored in a cool and dark place.

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

#### 5.3.1 Measuring Equipment and HPLC Conditions:

Instrument: HP 1100  
 Injector: HP 1100  
 Column: Phenomenex, Luna C18 (2), 5 µm, 15 cm, 0.46 cm i.d. or equivalent  
 Injection volume: 50 µL  
 Oven temperature: 40° C  
 Mobile phase: A: Water/ACN (90/10, v/v)+ 0.1 mL acetic acid per litre  
 B: Acetonitrile + 0.1 mL acetic acid per litre

Time Table	0 min	11.1 % B
	10 min	11.1 % B
	10.1 min	90 % B
	15 min	90 % B
	15.1 min	11.1 % B
	19 min	11.1 % B

Stoptime: 19 min  
 Flow (column): 1.0 mL/min  
 Flow (into MS): 0.15 mL/min  
 Retention time: Olefin-metabolite: approx. 4.6 min  
 Hydroxy-metabolite: approx. 5.5 min  
 Imidacloprid: approx. 9.1 min

NOTE: Conditions may be adapted for other HPLC-MS/MS systems.

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### 5.3.2 MS/MS-Detection

The experiments were performed on a triple-quadrupole mass spectrometer system, fitted with an electrospray interface operated in the positive ion mode under MRM conditions. The mass spectrometer was tuned by infusing a standard solution of 0.5 mg/L imidacloprid and its metabolites (dissolved in water/acetonitrile 8/2 + 0.1 mL acetic acid per L) at a flow rate of 10-20  $\mu\text{L}/\text{min}$ . Mass axis calibration was done by infusing a polypropylene glycol 3000 solution. Unit mass resolution was established and maintained in each mass resolving quadrupole by maintaining a full width at half-maximum of between 0.8 and 1.0 DA. After tuning and calibration, optimal collision-activated dissociation (CAD) conditions for fragmentation of imidacloprid and its metabolites were determined. These experiments were performed with nitrogen as collision gas with a collision offset of -19 eV for imidacloprid, -21 eV for the hydroxy-metabolite and -13 eV for the olefin-metabolite and at an approximate collision gas thickness of  $1.46 \times 10^{15}$  atoms/cm<sup>2</sup>. Nebulizer gas is set at 1.48 L/min, curtain gas is set at 1.44 L/min collision gas is set at 0.87 L/min and turbo gas is set at 6.0 L/min.

Detector: Triple Quadrupole LC-MS/MS Mass Spectrometer, e.g.  
Perkin-Elmer Sciex Instruments  
API 365, Apple™ Macintosh System® 8.1

Interface: Electrospray Turbo Ion Spray  
Potential: + 4400 V  
Temperature: 400 °C  
Nebulizer gas: Nitrogen 5.0 (99.999 % purity), 1.48 l/min  
Curtain gas: Nitrogen 5.0 (99.999 % purity), 1.44 l/min  
Turbo gas: Nitrogen 5.0 (99.999 % purity), 6.0 l/min

Scan type: MRM (Multiple Reaction Monitoring Mode)

Polarity: Positive

Collision gas: Nitrogen 5.0 (99.999 % purity), 0.87 l/min

Mass spectrometer operating parameters:

Compound	Precursor Ion Q1 Mass (amu)	Product Ion Q3 Mass (amu)	Dwell Time (msec)	Collision Energy (eV)
Olefin-metabolite (37)	256 <sup>#</sup>	238	250	-13
Olefin-metabolite (35)	254	236	250	-13
Hydroxy-metabolite (37)	274 <sup>#</sup>	191	250	-21
Hydroxy-metabolite (35)	272	191	250	-21
Imidacloprid (37)	258 <sup>#</sup>	211	500	-19
Imidacloprid (35)	256	209	500	-19

<sup>#</sup>: The CI 37 containing ions were detected to calculate the isotope ratio

NOTE: Different MS/MS-instruments or instrument parameters may result in different ion transitions and different relative intensities.