

Hazards of pesticides to bees – 10<sup>th</sup> International Symposium of the ICP-Bee Protection Group

# Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize

Pistorius, J. 1\*, Bischoff, G. 2, Heimbach, U. 1, Stähler, M. 2

Julius Kühn-Institut:

- <sup>1</sup> Institute for Plant Protection in Field Crops and Grassland,
- <sup>2</sup> Institute for Ecological Chemistry, Plant Analysis and Stored Products Protection

### Abstract

In spring 2008 a high number of bee poisoning incidents was recorded during sowing of maize in the Upper Rhine valley and in South Bavaria near Passau. More than 11.500 honey bee colonies from about 700 beekeepers in the Upper Rhine valley showed symptoms of insecticide poisoning. The reason for the poisoning was the abrasion of dust from maize seeds treated with the insecticide Poncho Pro (a.s. clothianidin) during the sowing process and blowing out of this dust containing the active substance into the environment with pneumatic sowing machines, resulting in contamination of nectar and pollen. The poisonings occurred in areas in southern Germany in which an eradication program for the quarantine pest *Diabrotica virgifera virgifera* was active and where clothianidin was used at a high rate (125 g a.s. /ha) on a large scale.

An exceptionally high amount of dust of up to 80 g per 100.000 kernels of maize was detected in some of the maize seed batches. The chemical analysis of dust, plant samples, bee samples, fresh pollen and bee bread confirmed the poisoning by clothianidin originating from treated maize seeds. No correlation with any bee pathogens was detected.

Keywords: seed treatment, drilling machines, neonicotinoid, clothianidin, dust, maize, drift, bee poisoning, honey bees

## Introduction

A high density of bee colonies in Germany is located in the Upper Rhine valley in Baden-Württemberg, due to the mild climate which promotes the overwintering of the colonies and a rapid development of the colonies in spring. Furthermore a high amount of bee attractive crops such as fruit trees and winter oilseed rape with short distances to other foraging possibilities like sweet chestnut, white fir and Norway spruce allow for good honey yield and excellent bee keeping conditions. At the beginning of April 2008, above-average overwintering losses of bee colonies of up to about 40% in some areas had been registered in the region, already before the first incidents of poisonings were reported in the last week of April 2008 during spring development of the colonies. High numbers of bee poisoning incidents were recorded during sowing of maize in the Upper Rhine valley and in parts of South Bavaria.

First reports of bee poisonings in a single municipality in the upper Rhine valley at end of April 2008 were followed within a few days by reports of some hundred beekeepers claiming bee damages, describing typical clinical symptoms of acute insecticide poisoning. Dying bees with obvious symptoms of intoxication like cramping, disoriented behaviour and abnormal wing movements were discovered in front of the hives and inside the hives, amounting up to several thousands of dead bees per day. Mortality remained at a higher level up to several weeks, resulting in weakened colonies. Some colonies showed only minor bee losses and only a slightly enhanced mortality, while other colonies showed severe damages; the scale of impact of the poisoning of colonies damage varied between 10-90%. Total losses of colonies were reported in only a few cases. Damages were not equally distributed in all local communities of the area; in some communities no bee poisonings were noticed. Since poisoning incidents happened on a large scale, after few days a temporal and spatial connection with the sowing of clothianidin treated maize was suspected and soon confirmed by residue analyses of the samples from incidents.

<sup>\*</sup>Tel.: +49 531 299 4525; Fax: +49 531 299 3008. E-mail: jens.pistorius@jki.bund.de

In the areas with bee poisonings maize seeds were treated with clothianidin for eradication of the western corn rootworm (*Diabrotica virgifera virgifera*), which is, according to the Directive 2000/29/ EG, classified as a quarantine pest by the EU. Compared to the normal use rate for wireworm control on about 5 % of the maize growing area in Germany (50 g a.s. clothianidin/ha), a high rate of clothianidin (125 g a.s./ ha) was used for the eradication purpose on large scale in the affected regions of southern Germany. The seed treatment Poncho Pro with the active substance clothianidin at the high rate had already been used in Germany on a smaller scale in 2006 and 2007, but no bee poisoning incidents had been reported. In 2008, although Poncho Pro dressed maize seeds were used in a larger area in the upper Rhine valley for eradication of *Diabrotica virgifera virgifera*, only some communities reported a high number, but other only a very low number or no bee poisoning incidents at all.

### Material and Methods

## Sampling, reporting and documentation of poisoning incidents

First samples of bees and plants were taken by the affected bee keepers. Samples were sent to the federal examination centre for bee poisoning incidents at the Institute for Plant Protection in Field Crops and Grassland, Julius Kühn-Institut (JKI).

As within a few days the extent of damage on a large scale became obvious, further sampling of bee and plant samples, documentation and initial collection of claims and reports were conducted by specialized local consultants for bee-keeping, experts from the beekeepers associations, departments from the Ministry of Food and Rural Land (MLR) and the Regional Councils of Stuttgart and Freiburg (RPS, RPF) and the plant protection services of Baden-Württemberg in cooperation with the beekeepers. Additional to residue analyses of bees, bee matrices and plants by the JKI also the state laboratory in Baden-Württemberg, Landwirtschaftliches Technologiezentrum, Augustenberg (LTZ) and a laboratory of Bayer CropScience carried out residue analyses. The consultants for bee-keeping or experts from the bee keepers association examined the colonies in order to estimate the damage and the extent of damage of the colonies.

# Analyses of plant and bee samples

Bee samples were screened for common bee parasites, like *Varroa*, *Nosema*, and amoeba. A visual inspection and microscopic examination of stomach contents and bee parts was conducted in addition to the fenotypical determination of the origin of the pollen in the body hair and corbicular loads.

Chemical analyses of samples of the poisoning incidents were conducted at the Institute for Ecological Chemistry, Plant Analysis and Stored Products Protection, Julius Kühn-Institut.

Screening and identification of about 200 different active substances in plant and bee samples were generally conducted with GC/MS [Trace DSQ II (Thermo Scientific)] and LC/MS/MS [Triple quadrupole mass spectrometer API 4000 QTRAP (Applied Biosystems MDS Sciex) coupled to a Shimadzu HPLC-system ], covering pesticides either authorized in Germany or in other countries of the E. U.

LC/MS/MS was used for the determination of clothianidin and methiocarb in the samples. For quantification the use of matrix-matched calibration with internal standard was necessary. The method was validated by conducting recovery experiments with bee and plant material. The mean recovery at the fortification level of 0.05 mg/kg was 120 % with a relative standard deviation of 2 %. The limit of detection was 0.5  $\mu$ g/kg for clothianidin in bee matrices and 0.3  $\mu$ g/kg in plant matrices and 1.0  $\mu$ g/kg for methiocarb in bee and plant matrices. Further details about the method will be published soon.

# Dust in the seed bags, abrasion of seeds

After the causal connection of the bee poisonings with sowing of maize became clear, seed lots were bought from the market and the amount of dust in the seed bags analysed. Originally sealed batches of maize seeds of different varieties and different insecticidal seed treatments were emptied carefully and sieved over a 6 mm sieve and the amount of dust of the whole bags documented. The size of dust particles was separated into the fraction of finer dust <0.5mm and coarse dust. Broken seeds were removed and not weighed.

Residues of the dust were analysed separately for fine and coarse dust particles using liquid chromatographymass spectrometry with elektrospray ionization (ESI). The LC-MS system consisted of a high-performance liquid chromatograph Perkin Elmer Series 200 and an API 2000 (Applied Biosystems MDS Sciex) with triple quadrupole mass spectrometric detection. Final determination is by LC. The analytes were separated with mobile phase gradient on Synergi Max RP 4 µm 75 mmx4.6 mm i.d. (Phenomenex) using an internal standardisation. The dust samples were extracted with acetone and diluted with internal standard. The limit of quantification for this special screening has been set at 0.1 mg kg-1.

## Results and Discussion

## Reports and observations of poisoning incidents

A varying extent of damage was observed at different apiaries. The on-site inspection of damaged colonies revealed that 25.3% of 12.174 damaged colonies from 736 beekeepers suffered damages less than 33%. Of the colonies 57.1% had a damage between 34% and 66%, 17.6 % were more than 66% damaged (MLR/LTZ, 2008). In Bayaria, approximately 460 colonies from 36 beekeepers reported bee poisonings in areas with Diabrotica eradication programme. The extent of damage to colonies and the level of contamination of an apiary was different due to the individual situation in the surrounding of the colonies; influenced for instance by the distance of maize sowing to flowering plants, the seed batch quality, wind conditions, the type of sowing machine and air outlet, and level of contamination of nectar and pollen, the attractiveness and foraging intensity on highly contaminated crops and the use of uncontaminated crops. During full bee flight in the first days after the beginning of poisonings, many dying and dead bees were found in front of the hives. Returning nectar foragers with symptoms of intoxication were noticed in front of the hives and while entering hives, passing on the contaminated nectar to other bees. During flowering of the contaminated crops, up to thousands of dead and dying bees were discovered daily during bee flight activity. In addition to these acute toxic effects colonies suffered further retarded effects after the end of spring flowering. Some beekeepers did not follow the official recommendation to remove all combs containing pollen from affected colonies. In these cases damages continuing up to several weeks after sowing of maize demonstrated that the contaminated pollen was stored as bee bread, subsequently causing the death of those bees consuming higher amounts of contaminated pollen, like nurse bees. Especially in the early morning dead and dying bees were found in front of these hives. Symptoms of damaged brood were observed, possibly due to poisoning by contaminated food, but also brood damages by the loss of adult and hive bees were observed as brood areas could not be maintained and undercooling of brood areas occurred. In general, colonies recovered after flowering of the contaminated crops had ended; before flowering of maize started, bee keepers raised concerns that residues systemically translocated into pollen of maize could lead to further intoxifications or poisonings.

Bees and bee matrices: The first visual inspection of the samples showed a rather small portion of pollen foragers in many of the bee samples. Analyses for bee diseases revealed that the incidents were not linked with bee diseases or parasites. The microscopic analysis of spores of *Nosema* spp. in 24 samples detected no spores, in 43 samples low amounts of spores (<5), in 15 samples a moderate number (10-30 spores) and in 3 bee samples a high number of more than 30 spores. The Analysis of *Nosema* spores indicated that both bees with and without *Nosema* were equally affected by the poisonings. Furthermore analyses of samples at the CVUA, Freiburg confirmed that the bee samples were not infected with viral diseases or other bee diseases. (Ritter, 2008, pers. comm.). A biotest of contact toxicity with bee samples and larvae of *Aedes aegypti* (n=70) was positive in 91.4%. In some of the bee samples, small coloured dust particles were discovered in the midgut. The fenotypical determination of the origin of the pollen in the body hair and corbicular loads demonstrated that the poisoned bees had foraged on a wide range of plant species and not only on one main crop.

Table 1 Poisoned bees analysed for residues

Range	Bees with clothianidin	%	Bees with methiocarb	%	_
n =	77	100,0	77	100,0	_
no residues	6	7,8	52	67,5	
0-1 μg/kg	1	1,3	0	0,0	
1-5 µg/kg	2	2,6	18	23,4	
5-10 µg/kg	25	32,5	6	7,8	
10-15 μg/kg	24	31,2	0	0,0	
15-20 µg/kg	10	13,0	1	1,3	
$20-40 \mu g/kg$	7	9,1	0	0,0	
$> 40 \mu g/kg$	2	2,6	0	0,0	
Total with residues	71	92,2	25	32,5	
Maximum μg/kg	212,2		18,5		

Chemical analyses of poisoned bees collected by the bee keepers revealed the presence of clothianidin in most bee samples, in fresh pollen collected with pollen traps and in bee bread of damaged colonies sent from the affected regions. The results of the analyses indicated that eight additional samples originating from regions where Poncho Pro had been used were clearly linked to spray applications with other insecticides.

The bee poisoning incidents were clearly linked with the abrasion of an insecticidal seed treatment during sowing of maize. Multiple background residues of further insecticides, fungicides and herbicides were also found in all bee and bee bread samples. In some of the bee and bee bread samples, also methiocarb was found indicating the origin of these active substances from treated seeds. Some of the maize seed lots treated with the high rate of clothianidin were additionally treated with methiocarb to repel birds. Methiocarb deriving from seed treatments was never linked with bee poisoning incidents before 2008 though being used for more than 20 years. The maximum rate of methiocarb treatment to seeds is 150 g a.s./ha. In regions with poisonings the rate of clothianidin was 125 g a.s./ha. In 6 bee samples no clothianidin was detected. In 2 of these bee samples which were sent several weeks after the initial damage, the storage conditions of the samples were not described, and clothianidin was not detected in bees, but in bee bread. In one sample, it was not possible to conclude or exclude a clear link with sowing as only the plant sample had a very low contamination with clothianidin, fipronil and a higher contamination with methiocarb but the bee sample was not contaminated. In another three samples no clothianidin but fipronil was found, possibly by use of imported seeds treated with fipronil.

 Table 2
 Residues of clothianidin and methiocarb in bee bread samples sent by beekeepers

Range	Samples with clothianidin	%	Samples with methiocarb	%
n =	20	100	20	100
no residues	9	45	2	10
0-1 μg/kg	3	15	1	5
1-5 µg/kg	6	30	1	5
5-10 µg/kg	1	5	7	35
10-15 μg/kg	1	5	5	25
15-20 µg/kg	0	0	1	5
20-40 μg/kg	0	0	2	10
$> 40 \ \mu g/kg$	0	0	1	5
Total with residues	11	55	18	90
Maximum μg/kg	15,5		83,4	

Analyses of 67 bee samples at the LTZ revealed 28.4% without detectable residues, 25.4% of the samples had 1-5  $\mu$ g/kg, 38.8% had 5-10  $\mu$ g/kg, 7.5% had 10-15  $\mu$ g/kg. No higher rates were detected. Some of the samples which had no residues were taken after the end of flowering, as damages were already decreasing or had stopped (Trenkle, 2009, pers.comm.). No detectable residues were found in analysis of 58 of 65 honey samples. In 7 honey samples, only low residues between  $1.1 - 2.3 \mu$ g/kg were detected (MLR, 2008).

Clothiandin and methiocarb were also found in pollen and bee bread. In 3 pollen samples collected with pollen traps 12.5, 5.4 and 26.4  $\mu$ g clothianidin/kg, and 27.9, 6.5 and 26.9  $\mu$ g/kg methiocarb were detected. 8 samples of bee bread had no residues of clothianidin and were not linked with sowing of maize. Clothianidin was not detected in one sample of bee bread, but in dead bees from the same colony. The presence of residues in pollen and bee bread confirms that pollen foragers successfully foraged and stored highly contaminated pollen in the combs. Pollen foragers were most likely less affected by acute poisoning during foraging activity compared to nectar foragers.

Analyses of 117 samples of LTZ, LUFA Speyer and Bayer CropScience (MLR/LTZ, 2008) revealed no residues in 65.8% of the bee bread samples, 6.0% had 1-5  $\mu$ g/kg, 5.1% had 5-10  $\mu$ g/kg, 6.8% had 10-15  $\mu$ g/kg, 0.9% 15-20  $\mu$ g/kg, 11% 20 -50  $\mu$ g/kg and 4.3 % had more than 50  $\mu$ g/kg clothianidin. The maximum was 77  $\mu$ g/kg clothianidin.

Due to the individual foraging behaviour, the pollen supply of the colonies usually consisted both of uncontaminated pollen and pollen contaminated with varying amounts of residues. It is most likely that especially crops directly neighbouring maize fields were strongly contaminated. Nectar and pollen collected from these field edges were highly contaminated and varying amounts of residues were present in food sources in the surroundings. Several pollen loads are used to fill one cell. It is most likely that individual pollen layers in a bee bread cell show a variation of the residue content, and also a variation between cells. As for a representative residue analysis of a bee bread sample several cells are needed, a partial contamination can not be detected. The analysis may therefore underestimate relevant residues in smaller bee bread fractions. As the pollen may be stored and consumed at a later date, retarded poisonings of bees can occur. This explains beekeepers reports that after rainy periods or periods of low pollen income increased but moderate numbers of bees were observed showing symptoms of poisoning or behavioural abnormalities. In general, after that flowering of directly contaminated plants had ended and other nectar and pollen became available colonies were able to recover well from the damages.

During flowering of maize, beekeepers were concerned that systemically translocated residues might cause new poisoning incidents, but only very few claims of poisoning incidents were reported from bee keepers in the region after sowing of maize. Some beekeepers claiming poisoning incidents suspected these damages were caused by residues of clothianidin in maize pollen. Residue analyses concluded no link with maize but poisonings by spray applications linked with different crops instead. The level of residues present in pollen of maize from plants and in pollen from pollen traps was in the expected range and covered by earlier risk assessment studies, indicating no risk for bees. In a monitoring programme of Bayer CropScience, 250 maize pollen samples had a mean of 3.4 µg/kg clothianidin, in 118 samples of pollen from pollen traps at the monitoring hives a mean of 1.1 µg/kg, in 36 bee bread samples a mean of 1.0 µg/kg was detected (Nikolakis et al., 2009). The residue levels present in pollen of maize were clearly lower compared to residues in pollen of flowering plants during sowing of maize. Three samples of fresh pollen from pollen traps had 1.7-3.0 μg/kg clothianidin. No further poisoning symptoms and no disturbance of colony development were observed during and after flowering of maize. Monitoring of damaged bee colonies in the upper Rhine valley did not show any adverse effects on bee health during flowering of maize. No further adverse effects on the colonies regarding overwintering strength, overwintering success and colony strength in spring 2009 were observed (Liebig et al., 2008). A fair overwintering with no unusual overwintering losses 2008/2009 was reported by the bee keepers from the regions affected by the poisonings in 2008.

<u>Plants</u>: Residues of clothianidin in plants that had received no deliberate insecticidal treatments were detected in apple flowers (n=7), dandelion flowers (n=2) and other wild flowers (n=11) and winter oilseed rape (n=15) collected in the proximity of fields. Clothianidin is also used as seed treatment of winter oilseed

rape. Therefore residues of clothianidin were also detected in samples of winter oilseed rape, but only high residues exceeding by far the background levels that may originate from systemic translocation in the plants, can cause the strong poisonous action.

Table 3	Plant samples with residues of clothianidin and methiocarb sent by beekeepers
rable 3	Figure Samples with residues of clountaintum and methodard sent by beekeepers

Range	Plant samples with clothianidin	%	Plant samples with methiocarb	%
n =	35	100,0	35	100,0
no residues	3	8,6	21	60,0
0-1 μg/kg	3	8,6	4	11,4
1-5 µg/kg	14	40,0	3	8,6
5-10 μg/kg	9	25,7	2	5,7
10-15 μg/kg	3	8,6	2	5,7
15-20 µg/kg	0	0,0	1	2,9
20-40 μg/kg	2	5,7	1	2,9
$>40~\mu g/kg$	1	2,9	1	2,9
Total with residues	32	91,4	14	39,4
Maximum μg/kg	47,8		43,0	

Most of the plant samples analysed contained residues of clothianidin and methiocarb. At the time of first plant samplings by the beekeepers the reason for the damages was not clear. Suspecting damages by spray applications, many beekeepers randomly took samples from flowering crops nearby, without knowing the link with sowing. As the documentation of some of the samples was incomplete, a detailed interpretation of the residue data is not possible for all plant samples. Nevertheless it is most likely that these samples underestimate the maximum levels of contamination that were present in the area, as sampling was not targeted to flowering crops neighbouring maize, but even low residues in flowers except winter oilseed rape indicate that drift of dust was the cause for the residues. Some plant samples were excluded from further analyses as no further gain of information could be expected, or the bee samples concluded no link with dust poisoning.

The possibility of emission of dusts containing active substances from dressed seeds during sowing of imidacloprid-treated maize was already shown by Greatti et al. (2003, 2006). Residues detected were in grass samples between 14  $\mu$ g/kg and 29  $\mu$ g/kg, in flowers between 22 and 59  $\mu$ g/kg (Greatti et al. 2006). In another trial with different varieties and seed treatments residues ranging from 22.4  $\mu$ g/kg to 123.7  $\mu$ g/kg were found on flowers, on grass residues ranging from 40  $\mu$ g/kg to 58  $\mu$ g/kg were found. High residues in plants samples were also confirmed by analyses of the LTZ Augustenberg (MLR/LTZ, 2008). Target-orientated, bee-attractive flowers from plants neighbouring maize fields were sampled, maximum residues of clothianidin found in apple flowers were 98.5  $\mu$ g/kg, in winter oilseed rape flowers 94.5  $\mu$ g/kg and 113  $\mu$ g/kg in dandelion flowers. One drift study carried out by LTZ examining the residues on winter oilseed rape fields after sowing treated maize in adjacent fields demonstrated residue values of about 100  $\mu$ g/kg on flowers in 1 m distance, reduced to about 70  $\mu$ g/kg in about 5 m distance.

# Dust in the seed bag

After the causal connection of the bee poisonings and the contamination of plants with sowing of maize became clear, seed lots were bought from the market and the amount of dust in the seed bags analysed.

A high variation was detected for the amount of dust in 82 different seed lots ranging from 2 to about 60 gram per 80.000 kernels (amount for 1 ha) with an average of 3.6 g fine dust and 4.9 g coarse dust. The coarse dust contained mainly larger plant particles (glumes) from seeds of maize which were treated together with the seeds but broken from the seeds. Fine dust mainly seems to appear if the quality of the coating process is not sufficient. The occurrence of glumes depends on cultivars, harvesting time and technique, and intensity of cleaning of seeds before the coating process. Often the glumes were broken in smaller particles

falling into the fine dust fraction. High amounts of dust were also detected in several batches of seed bags of maize by the LTZ Augustenberg (MLR/LTZ, 2008).

## Fine and coarse dust in maize seed bags

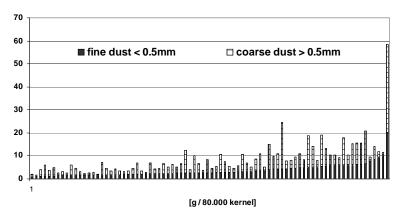


Figure 1 Fine dust <0.5 mm and coarse dust > 0.5mm after slightly sieving of whole seed bags with 6 mm mesh size (different batches, collected from the German maize seed market)

The dust of those seed batches treated with clothianidin (often also with other fungicides and methiocarb) was analysed for clothianidin residues. High residue contents were detected in the dust with significant higher rates in the fine dust compared to the coarse dust (Tab. 4). Lower residues in coarse dust can be explained by more plant material in this dust fraction which dilutes the percentage of clothianidin. Dust treated with the higher rate of clothianidin had significant higher residues. But clothianidin residues in fine dust were not significantly affected by the amount of fine dust per seed bag detected. There is a tendency that higher rates of coarse dust in seed bags of maize reduce the residue content which might be explained by the higher amount of plant material which partly also falls into the fine dust fraction and which dilutes the residue content. Coarse dust containing larger amounts of plant material is less contaminated with clothianidin.

**Table 4** Clothianidin residues (in %) in dust (a: fine dust, b: coarse dust) sieved from 50 different seed batches of maize treated with Poncho or Poncho Pro. Sd: standard deviation.

		dust < 0.5mm clothianidin		dust > 0.5mm clothianidin	
clothianidin / kernel		in %	sd	in %	sd
N = 20	0.5 mg (Poncho)	18.5 a, A	5,7	11.4 a,B	3,5
N = 30	1.25 mg (Poncho Pro)	28.2 b, A	8,6	14.7 b, B	5,1
a: fine dus	t < 0.5 mm in g				
N = 12	1.2 - 1.75	23.8	6,4	16.2	6,6
N= 15	1.9 - 3.0	27.0	11,4	12.7	3,6
N = 23	> 3	23.2	7,8	12.9	3,7
b: coarse d	lust > 0.5 mm in g				
N= 17	0.5 - 2.7	27.9	6,4	15.5	5,5
N= 15	2.7 - 4.7	24.8	10,8	11.8	4,8
N= 18	> 4.7	20.4	8,0	12.7	3,3

t-test, raw data arcsin transformed (significant differences p<0,02; a,b vertical, A,B horizontal)

First analyses in 2008 showed that seeds from different crops vary in the amount of dust. Whereas maize seed batches generally contained dust particles of varying but high amounts, only very low amounts of dust were found in crops like sugar beet or winter oilseed rape. (Heimbach, unpublished). Size and structure of different seeds allow different seed treating techniques and a coating enclosing the whole seed.

The specific sowing technologies for different crops may result in varying amounts of dust blown out, depending on technical solutions by the manufacturers of sowing machines.

### Conclusion

The high number of poisoning incidents in Baden-Württemberg and Bavaria in spring 2008 could clearly be linked to the sowing of maize and the abrasion and emission of dust containing the active substance clothianidin. Emission and drift of considerable amounts of dust particles may pose a risk for honey bees when bee toxic substances are used for seed treatments.

Clothianidin was detected in the chemical analyses of poisoned bees collected by the bee keepers but also in samples of plants, fresh pollen and bee bread after the high rate of clothianidin of 125 mg a.s./ha had been used. Some maize seed bags with exceptionally high amounts of dust were found. Dust containing active substance was emitted with pneumatic sowing machines resulting in drift of dust and contamination of bee attractive plants at the time of main nectar flow.

To enable a safe sowing of seed treated crops in the future, the improvement of seed quality and a reduction of emission due to actions taken by seed breeding companies, seed treating companies, corn drilling machinery industry and chemical industry is necessary. The technique of the seed treatment varies for different crops, also the structure of seeds is different. This may cause differences in the amounts of dust. First results show that seeds from different crops vary in the amounts of dust in the seed bag. Technical solutions for avoiding the formation of loose dust must be established to remove all unwanted dust particles before, during and after the coating process. The glume particles originating form the seeds of maize need to be removed as far as possible before the seeds are treated. Proper coating systems and appropriate stickers must be used to ensure that only a minimum of dust can be abraded during handling and sowing of maize seeds. Furthermore, the coating process must guarantee that during handling, transport, and sowing no new dust should be generated. A quality check of treated maize seeds needs to be established after the coating process. The Heubach Dustmeter is in use for 2009 and seems to be suitable for this purpose. Maximum Heubach values need to be defined which ensure a minimum of dust during sowing.

Concerning the future of insecticidal seed treatments, appropriate risk mitigation measures for potential abrasion and dust generation need to be established for crops with a potential of considerable amounts of dust. In addition to an improved seed quality the emission needs to be stopped by technical solutions for sowing machines as far as possible, regardless of the substance. Promising appropriate first technical solutions for drift reduction have been developed, are inexpensive and have proven to be effective to reduce drift for most maize sowing machines. Sowing machines used today are mostly precision pneumatic planters with vacuum singling. In case of maize, especially pneumatic single-seed machines seem to have a high emission potential. The emission of these dust particles with pneumatic single-seed drilling technology needs to be reduced. Most of those machine types centrally blow the air of all rows upward or sideward resulting in a contamination of neighbouring area and therefore are vulnerable to wind drift. Changing the direction of the air from the outlets of the seeders and reducing wind speed seems to be a promising technique to reduce drift. A method was established in the JKI to measure drift reduction due to different modifications of the air exhaust. In autumn 2008 the sowing machines of all well known manufacturers were tested by the Institute for Application Techniques in Plant Protection. For several types of seeders modifications of the air exhaust now directed to the soil were constructed by the manufacturers. The modified setups were tested; those which proved a drift reduction of at least 90 % were registered in the JKI-list "drift reducing maize sowing machines" (Rautmann et al., 2009).

Such drift reduction setup change is compulsory for sowing insecticidal seed treated maize in Germany in 2009.

Furthermore, within a risk assessment the possible amount and drift of dust, and the potential hazard of dust for honeybees should be taken into account. Hazard quotient (HQ) values are in use to describe the risk for honeybees caused by spray applications. The HQ approach or TER- calculations may possibly be used for risk assessment but must be adapted from spray applications to the risk of dust emission, as the allocation, dispersion and deposition of dusts may be different from sprays. Only a fraction of the total active substance is blown out during sowing with dust, but the higher portion of this dust is presumably depositing within a short distance, within few meters of the sowing machine. For the risk assessment, trials on dust emission were formerly conducted but these did not indicate a considerable risk for bees. Only clean seed batches seem to have been used for these trials. To estimate possible adverse effects to honey bees, the development of appropriate new approaches of study designs are required to cover sowing scenarios and generate basic data necessary for improving the effectiveness of the established risk assessment schemes.

#### References

- 2. Ministerium für Ernährung und Ländlichen Raum, Abschlussbericht des MLR über Beizung und Bienenschäden vom 17. Dez. 2008, http://www. Landwirtschaft-bw.info/ [accessed 14.4.2009].
- Ministerium für Ernährung und Ländlichen Raum, Press release 104/2008: "In Honigproben wurden keine Rückstände des Maisbeizmittels Clothianidin festgestellt", http://www.mlr.baden-wuerttemberg.de/ [accessed 12.1.2009].
- 4. Alexander Nikolakis, Andrew Chapple, Reinhard Friessleben, Paul Neumann, Thorsten Schad, Richard Schmuck, Heinz-Friedrich Schnier, Hans-Jürgen Schnorbach, Ralf Schöning, Christian Maus (2009): An effective risk management approach to prevent bee damage due to the emission of abraded seed treatment particles during sowing of seeds treated with bee toxic insecticides. Julius-Kühn-Archiv 423, 160 p.
- Liebig G., Kustermann T., de Craigher D., Bienenmonitoring w\u00e4hrend und nach der Maisbl\u00fcte im Rheintal 2008. Unpublished report (2008).
- Greatti M., Barbattini, R., Stravisi A., Sabatini, A.-G.,Rossi, S., Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing. Preliminary results, Bulletin of Insectology 56 (1): 69-72, (2003).
- Greatti M., Sabatini, A.-G., Barbattini, R., Rossi, S., Stravisi A., Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho ® dressed seeds, Bulletin of Insectology 59 (2): 99-103, (2006).
- Rautmann D., Osteroth, H.-J., Herbst, A., Wehmann, H.-J., Ganzelmeier, H., 2009, Prüfung abdriftmindernder Sägeräte. Journal für Kulturpflanzen 61(5),S. 153-160. http://www.jki.bund.de/cln\_045/nn\_806762/DE/pressestelle/Presseinfos/2009/140109\_\_maissaegeraet.html.
- Pistorius J., Bischoff, G., Heimbach, U., 2009: Bienenvergiftungen durch Wirkstoffabtrieb von Saatgutbehandlungsmitteln w\u00e4hrend der Maisaussaat im Fr\u00fchjahr 2008. Journal f\u00fcr Kulturpflanzen 61(1). S. 9-14.

# Bee poisoning caused by insecticidal seed treatment of maize in Germany in 2008

Rolf Forster

 $Federal\ Office\ of\ Consumer\ Protection\ and\ Food\ Safety\ (BVL),\ Department\ 2\ Plant\ Protection\ Products,\ Messeweg\ 11/12,\ 38104\ Braunschweig$ 

Fon: +49 531 299 3610, Fax: +49 531 299 3005, E-mail: rolf.forster@bvl.bund.de

### Abstract

<u>Background</u>: In late April and early May 2008 a bee mortality occurred in parts of South-West Germany, which affected approximately 12,000 colonies of bees, some of them substantially. Immediately after this became known, an intensive search for the causes of these incidences was started.

<u>Results</u>: Very soon, maize seeds which had been treated with the insecticidal substance clothianidin were suspected as a possible cause. Only two weeks later a clothianidin poisoning was confirmed by the JKI. On May 15, 2008 the BVL-authority ordered suspension of the authorisation of a number of insecticidal seed