Prior to an adaptation of sowing material, the question of the risks posed by sowing dusts to honey bees remains. In France, a dedicated risk assessment has recently been performed for two PPPs to be used as seed coating[3]. Exposure of bees was assessed from dedicated experimental data on dust emission from the coated seeds according to high quality standard. The amount of active substance emitted was determined and used as an application rate estimate in a hazard quatient calculation, further compared to Directive 91/414/EEC trigger and by comparing the drift dose rate on dusts to acceptable exposure levels in tunnel testing. Due to the nature of the risks related to a sowing event, contact toxicity value was preferred. This risk assessment lead to conclude to acceptable acute risks for the products evaluated. Nevertheless, such an assessment may probably be improved and remains a precondition to routinely implemented controls of coating quality, through e.g. dust emission/abrasion tests.

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[1] Commission d'étude de la toxicité, des produits antiparasitaires et supports de culture, procès verbal de février 2004 (http://agriculture.gouv.fr/IMG/pdf/avisctweb200401.pdf)
[2] Greatti M., Barbattini R., Stravisi A., Sabatini A. G. and Rossi S., 2006. Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho dressed seeds. Bulletin of insectology 59 (2): 99-103.
[3] AVIS du CES relatif à une demande d'autorisation de mise sur le marché de la préparation Cruiser à base de thiaméthoxam, de la société Syngenta Agro SAS, dans le cadre d'une procédure de reconnaissance mutuelle.
Avis du CES relatif à une demande d'autorisation de mise sur le marché de la préparation Poncho Maïs à base de clothianidine, de la société Bayer Cropscience France, dans le cadre d'une procédure de reconnaissance mutuelle (http\\www.afssa.fr).

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

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Abstract

In spring of 2008, a bee incident occurred in the Upper Rhine Valley (Germany) during drilling of corn: bees were exposed to dust from abraded particles of the seed-coating containing the insecticide clothianidin. An inspection of drilled seed batches for resistance to abrasion and a geographical correlation analysis between specified seed batches and reported bee damages revealed that the incident was caused by improperly dressed batches of corn seeds with excessive abrasion of seed treatment particles which were subsequently emitted via the outlet air stream of the pneumatic drilling machines. Concerns raised by local beekeepers regarding effects on bees from foraging in seed-treated corn fields during bloom could be dispelled by a large-scale survey of clothianidin residues in pollen from the treated crop and an accompanying monitoring of bee hives exposed to flowering corn fields. In order to ensure the bee safety of seed-dressing products, technical improvements of seed treatment quality and drilling technology were developed resulting in a minimization of formation and emission of dust from abraded seed treatment particles. The efficacy of these improvements was proven in field trials.

Keywords: seed treatment, drilling machines, corn, clothianidin, dust, honey bees

Introduction

In late April and early May of 2008, numerous cases of increased bee mortalities were recorded in the Upper Rhine Valley (SW Germany). Typically, the affected bees showed symptoms of acute intoxication, in most cases these effects were seen in adult bees only. Approximately 11,500 bee hives were affected. The investigation of the incident was started by regional and Federal authorities immediately after the first records of conspicuous mortality. From the beginning, there were indications which linked the increased mortalities with the drilling of corn, which took place simultaneously in the affected region. In dead honeybees and samples of vegetation adjacent to drilled corn fields, residues of clothianidin were detected. Clothianidin is a neonicotinoid insecticide contained in the seed-dressing product Poncho Pro® (Clothianidin FS 600, 1.25 mg a.s./kernel), which is applied as a seed-dressing product to corn seeds and was used in the Upper Rhine Valley for control of the western corn rootworm (*Diabrotica virgifera*), an economically devastating pest in corn. Some farmers in the affected area reported unusually high amounts of dust in the bags of treated corn seeds and the emission of red dust during the drilling of these seeds. These reports provided indications that dust from abraded particles of the seed-dressing, which contained the intrinsically bee-toxic clothianidin, was released during the drilling process with the outlet air of pneumatic drilling machines and deposited on flowering, bee-attractive crops and weeds in adjacent vegetation strips and fields where bees were exposed during foraging.

A coincidence of several worst-case factors aggravated the impact of this excessive dust emission: the patchy landscape structure of the Upper Rhine Valley where many small-sized corn fields are located in a diverse agricultural landscape with canola fields, orchards, and other bee-attractive crops, the unusual climatic conditions in the year 2008 due to which the corn drilling and the flowering season of some crops like canola, several orchard crops, and others took place simultaneously, and dry, windy weather during the drilling season, which enhanced formation and drift of dusts.

This paper presents in its first part the results of a detailed analysis of the incident. A basic understanding of the factors causing this incident was seen as the key prerequisite to identify appropriate measures to reliably prevent a repeat of such accidents. This part likewise addresses potential risks posed by the systemic nature of the insecticidal component of the seed treatment product Poncho Pro® to honeybees. In response to massive concerns raised by the local beekeeper community, a residue survey was performed on corn pollen which was accompanied by a monitoring exercise of bee colonies which were installed on three locations within the Upper Rhine valley and regularly inspected. In the second part, this paper summarizes the outcome of a joint research initiative of seed-breeding companies, the drilling machinery industry and the agrochemical industry aiming at the development of appropriate technical solutions to ensure safety of seed-dressing products for honeybees and wildlife. Finally, the results of field trials conducted in order to evaluate the effectiveness of the developed optimizations are presented, and an exemplary bee risk assessment under consideration of the described mitigation measures is outlined.

Results and discussion

Investigation of the Incident

<u>Geographical analysis of correlation</u>: A quantitative analysis was performed with the goal to substantiate or disprove the assumption that an excessive emission of abraded seed treatment particles was the key factor causing the bee incident. In an interview survey, farmers had reported that during the sowing process considerable quantities of dust were generated by the sowing machines, and that this dust had been visibly emitted in the environment with the outlet airstream of the pneumatic drilling machines. Dust subsequently deposited also onto bee forage plants.

Georeferenced data on bee damage and clothianidin residue detects were compared with data on regional sales of Poncho Pro® treated seeds and the seed treatment quality (e.g., resistance to abrasion in standardized laboratory tests), as well as the drilling machineries used. Furthermore, data on land use (e.g., occurrence of corn and canola fields) and land cover (e.g., riparian zones of water bodies) were used, e.g., to characterize the occurrence of bee forage plants. Data were obtained from the Ministry of Food and Rural Land (MLR) and the Regional Councils of Stuttgart and Freiburg (RPS, RPF), and from laboratories of Julius-Kühn-Institut, Braunschweig (JKI), Landwirtschaftliches Technologiezentrum, Augustenberg (LTZ), and Bayer CropScience. Data processing was done in close cooperation with MLR and RPS.

The obtained data set covered the entire Upper Rhine Valley and the Lake Constance region. Information about dust formation during handling and sowing, the Poncho Pro® treated seed varieties (and batches) applied, the types of drilling machines used, as well as the occurrence of bee damage in the relevant area were surveyed by interviews of local farmers.

The analysis of local farmer reports (covering about 2,600 ha in the Upper Rhine Valley and about 600 ha in the Lake Constance region) indicated that in a number of cases poorly treated batches of Poncho Pro® treated corn seeds were sown (here called 'deficient batches') with pneumatic drilling machines resulting in bee damages in the surroundings. In some cases where seed varieties of appropriate seed treatment quality were applied with pneumatic drilling machines no bee damages were recorded, although bee hives and bee forage plants were present in the vicinity of the applied fields. A few cases where "deficient" batches were drilled with machinery of low dust emission potential (here: mechanical drilling machines) did not result in bee damage (presence of bees were confirmed for the respective local areas) indicating that the exposure can be reduced to acceptable limits by an appropriate application technology.

At the scale of the Upper Rhine Valley, a geographical comparison between the density of corn fields drilled with Poncho Pro® treated corn seeds (no differentiation of varieties or seed varieties) and the occurrence of bee damage shows (Figure 1), that in 33 municipalities no bee damage was recorded despite Poncho Pro® treated seeds had been drilled in significant amounts. Since the landscape of the Upper Rhine Valley is characterized by small-scaled and diverse cropping structures and other land uses (e.g., meadows, grassland with fruit trees, wood, etc.) and likewise contains varieties of areas with shrubs vegetation (e.g., along water bodies), it is reasonable to assume that bee forage was generally available. Therefore, it is highly unlikely that bees should not have foraged specifically in these regions since the entire valley is densely populated with bee hives. Likewise, in the Lake Constance region, in only 1 of 35 municipalities where Poncho Pro® treated seeds had been sown, a bee damage was reported. An effect of corn drilling technology could not be analyzed on this scale, as (standard) pneumatic drilling machines were predominately used across the whole region (applies also to the Lake Constance region).

Figure 1 Maps of the Upper Rhine Valley showing the use density of Poncho Pro®-treated corn seeds (left) and the abundance of cases of bee damage (right) on municipal scale.

From this geographical correlation analysis it can be concluded that the recorded bee damages are not related to the use of Poncho Pro® treated corn seeds per se but to the use of “deficient” batches of certain seed varieties which resulted in an excessive emission of abraded seed treatment particles and, by depositing on adjacent bee forage, in critical exposure levels for honeybees.

The relationship between seed treatment quality and the abundance of bee damage was further investigated in a two-step approach: In the first step, regional sales data of specified Poncho Pro®-treated corn seed varieties were correlated with recorded bee damage. This analysis was conducted on municipal level to minimize uncertainty due to bee activity radius and cross-border use of batches. In the second step, abrasion resistance of the analyzed seed batches was determined in standardized laboratory tests (see: Determination of the abrasion resistance of the final seed coating) and related to the correlations obtained in the first step.

In figure 2, the correlation coefficients between the use density of PonchoPro® treated seeds and the abundance of records of bee damage in the same municipality are shown for the top ten seed varieties regarding use densities across the entire Upper Rhine Valley (in decreasing order, i.e. variety 1 has the highest overall use density). These top ten seed varieties cover >75% of the market. The red bars in Figure 2 show the correlation between absolute use densities of the respective seed variety and the abundance of bee damages in the same municipality. A positive correlation indicates that batches of the respective seed variety had received an improper seed treatment. The green bars show the correlation between the market share of a variety and the abundance of bee damages in the same municipality. Negative correlations indicate that the used batches of the respective seed variety had received an adequate seed treatment. Although the simultaneous use of different seed varieties in the same locality and the heterogeneity of a variety regarding the seed treatment quality of different batches substantially increased the data variability, significant correlations could be detected for 9 out of the ten evaluated seed varieties indicating the high relevance of the investigated parameter, i.e. the seed treatment quality.

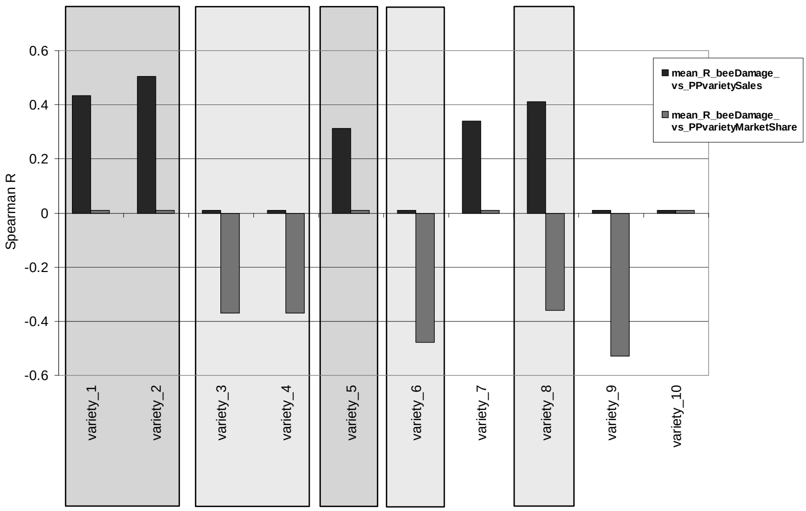


Figure 2 Spearman rank order correlation coefficient R (bars) and results of abrasion resistance tests (rectangles) for batches of corn seed varieties used in the Upper Rhine Valley in 2008. The red bars show the correlation coefficient for the regional use density of a variety and the abundance of bee damage records, the green bars show the correlation coefficient between the market share of a variety and the abundance of bee damage records. The red shaded boxes specify seed variety batches that have shown low resistance against abrasion in standardized laboratory tests, the green shaded boxes indicate tested batches of good seed treatment quality (in agreement with user reports).

The results of the abrasion resistance tests in the laboratory and the user reports on seed treatment quality were highly consistent. The abrasion resistance properties of the investigated batches of several relevant seed varieties are sketched in to figure 2. For seed varieties which correlated positively with the abundance of bee damages, “deficient” batches (red shaded boxes) were identified, whereas varieties which correlate negatively with the abundance of bee damage consistently showed appropriate seed-dressing quality (green shaded boxes). On first glance, seed variety 8 displays contradictory data. However, the use density of this variety strongly correlated with the use density of seed variety 2. Variety 2 contained “deficient” batches and had a significantly higher market share than variety 8. Accordingly, the negative impact of variety 2 explains the positive correlation result for variety 8 regarding use density. The negative correlation between market share and abundance of bee damages for variety 8 (green bars) is consistent with the finding of good abrasion resistance for the investigated batches of this variety.

According to similar data analysis, the single bee incident recorded in the Lake Constance region was most likely also linked with the use of a seed variety with batches of deficient seed treatment quality.

Therefore, the consistent correlations obtained for the relationship between the use density of PonchoPro®-treated seed with “deficient” batches and the abundance of bee damage strongly suggest that an improper seed treatment in combination with the use of the standard pneumatic sowing equipment was the main reason for the bee incident in the Upper Rhine Valley.

Pollen residue survey and hive monitoring exercise - effects of exposure of bee colonies to Ponchopro® treated corn pollen in the upper Rhine valley: Clothianidin is a systemic compound which translocates from the seed surface into the growing plant. Traces of residues may also be found in bee-relevant matrices from treated plants like pollen. In response to the incident during corn drilling, beekeepers raised the concern that bees might encounter systemic residues of the compound via pollen during the flowering period of the corn and that they could thereby be exposed a second time to harmful levels of clothianidin. In order to address these concerns, a large scale monitoring project was conducted in summer 2008. It basically consisted of two parts:

1. Sampling and residue analysis of pollen from treated corn on 50 fields at 5 different locations across the Upper Rhine Valley.
2. Bee health monitoring: on three of the residue sampling sites, bee hives were set up next to treated fields and surveyed for potential effects (conducted by Dr. G. Liebig, University of Hohenheim).

The five locations for pollen sampling were chosen with the focus on where bee incidents had been recorded during the corn drilling season (for distribution of sampling locations see Figure 3). At each of these locations, five corn fields were selected which were grown from PonchoPro®-treated seeds. From each of these fields, five individual corn pollen samples were taken from the crop during flowering period, so in total 250 samples were taken and subsequently analyzed for residues of clothianidin.

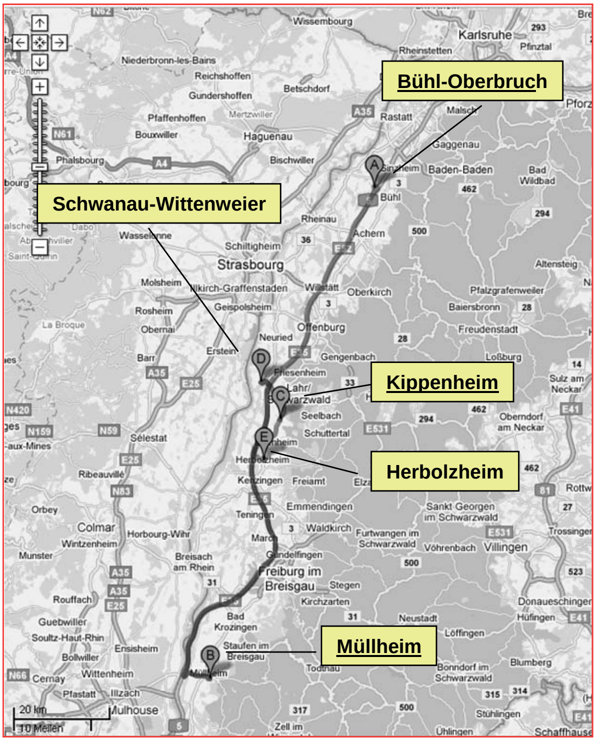


Figure 3 Location of the pollen sampling and the bee hive monitoring sites in the Upper Rhine Valley. Locations with underlined names are both pollen sampling and monitoring sites.

Residue levels in pollen sampled from the crop ranged from < 0.3 µg/kg (Limit of Detection) to 10.4 µg/kg clothianidin, with a mean residue level of 3.4 µg/kg.

For the bee colony monitoring exercise, 15 bee hives of different types, ages and constitutions were set up next to one of the monitoring fields at each of three of the sampling sites (Müllheim, Kippenheimweiler and Oberbruch, see Figure 3) shortly before the corn started to bloom. These colonies were closely monitored for their development and their health condition during the flowering period of corn and until the beginning of the overwintering season. The monitoring colonies developed well and no indications of an adverse effect related to an exposure to harmful chemical residues were found (Liebig et al. 2008). [1]

Along with the hive assessments, samples of hive matrices were taken for residue analysis. In samples of pollen from pollen traps at the monitoring hives, residue levels between < 0.3 µg/kg (LOD) and 11.4 µg/kg clothianidin were found (mean: 1.1 µg/kg, 118 samples). Samples of bee bread from the monitoring hives contained residue levels between < 0.3 µg/kg (LOD) and 3.3 µg/kg (mean: 1.0 µg/kg, 36 samples). The proportion of corn pollen in the pollen traps attached to the hives was very variable between different exposed colonies; on average, the share of corn pollen in the overall collected pollen was 22%, however, some colonies collected virtually no corn pollen, whereas others intensely foraged in the crop. The colony with the highest proportion of corn pollen among the foraged pollen collected 80% corn pollen (LIEBIG et al. 2008). [1]

No residues were detected in samples of dead bees from the monitoring hives with exception of two out of 38 sub-samples which showed a residue level of 1.2 µg/kg.

The residue levels found in pollen were consistent with previous findings in regulatory studies submitted for the national authorization (unpublished data); likewise the absence of any adverse effects in the exposed colonies confirms the conclusion of the regulatory risk assessment, and is consistent with the finding from several previous higher tier studies that exposure of bee colonies to dietary concentrations of clothianidin up to at least 20 µg/kg does not cause any adverse effects (Schmuck & Keppler 2003).[2]

From the pollen residue survey and the bee hive monitoring it can be concluded that systemic residues of clothianidin in corn pollen from Poncho Pro®–treated plants do not pose a risk to bee colonies.

As it was outlined in the previous chapters, the key conclusions that could be derived from the incident analysis were that the bee incident was caused by exposure of bees to abraded seed treatment particles from improperly treated seed batches, and that there is no risk from systemic residues in corn pollen of seed-treated plants. As a consequence of this, effective risk mitigation measures have to focus on two core aspects:

- **Seed treatment quality:** optimization of adhesivity of seed treatment products on treated seeds in order to reduce abrasion.
- **Seed drilling technology:** minimization of emission of abraded seed treatment particles to off-crop habitats.

Development and effectiveness of these mitigation measures are outlined in the following chapters.

Improvements in seed treatment quality and drilling technology

<u>Optimization of seed-dressing qualities</u>: As shown in the analysis of the bee incident in the Upper Rhine Valley, the quality of the seed coating is one of the key factors in avoiding contamination of the environment through abrasion of dust particles containing active ingredients from the seed treatment coat. Seed treatment in general is the process of applying fungicidal and/or insecticidal seat-dressing products onto various types of seeds. Today, the majority of seed treatment products or mixtures are applied as liquid slurry on seeds.

<u>Factors influencing seed coating quality</u>: The main factors influencing the quality of the seed coating in terms of dustiness / abrasion resistance are:

1. the quality of the seeds before the actual seed treatment process,
2. the technical and chemical composition of the used seed treatment formulation,
3. the employed seed treatment machinery and
4. the application recipe

- **Quality of seeds** before the actual seed treatment process: The most important factor is seed cleaning before treatment of seeds. Seed should be free of any organic dust particles as these will greatly affect the dustiness of the treated seeds at a later stage. As any movement of untreated seed will generate dust, an adequate aspiration system is important to remove all dust particles before the seed enters the seed treatment machine.
- **Formulation:** The quality of formulation of the seed treatment products used plays an important role. The main parameters are besides the particle size of solids (i.e. active ingredients, pigments etc.) the content of appropriate polymers (so called “stickers”) in the formulation to enhance the intrinsic adhesiveness.
- **Seed treatment machinery:** Corn seed is commonly treated with modern batch treaters as they offer high flexibility for adjustment and fine tuning of the treatment process according to seed type, seed quality and application recipe used.
- **Application recipe:** Besides the factors mentioned above, the final recipe of the final seed treatment slurry is amongst the most important factors influencing the final quality of the seed coating. Depending on market requirements usually a combination of different seed treatment products (fungicides and insecticides) at varying application rates are applied. Thus, application recipes are often complex and the total amount of products to be applied can vary significantly. In order to ensure a good adhesion of these products on the seed the addition of supplementary and appropriate adhesives (film-coatings) to the final seed treatment slurry is mandatory.

As shown in Figure 4, the addition of adhesives can significantly reduce the abrasion of dust from treated seeds.

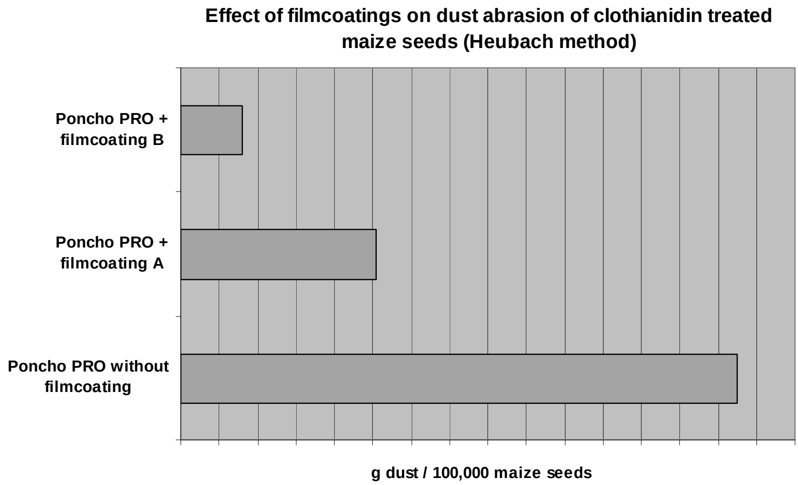


Figure 4 Effect of various film-coating products on the dust abrasion of clothianidin (Poncho Pro®) treated corn seeds

Depending on the seed type, the seed treatment products and their combination, the right adhesive at the optimum application rate has to be chosen to generate treated seeds with a high resistance against abrasion. As the surface properties and the geometry of different seed types (corn, canola, cereals, cotton, sunflower, vegetables, etc.) differ significantly, specific adhesives are designed for each seed type.

<u>Determination of the abrasion resistance of the final seed coating</u>: In order to quantitatively measure the abrasion resistance of treated corn seeds, the Heubach dust abrasion test has been identified as a viable test method which allows best for standardization of dust abrasion measurements within the seed industry, the crop protection industry and independent laboratories, including authorities. Ready-to-use Heubach - dustmeter equipment is commercially available (Figure 5).

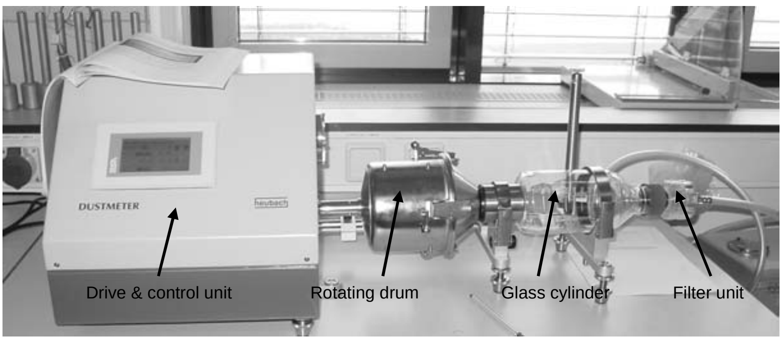


Figure 5 Heubach dustmeter equipment

Moreover, as the Heubach - dustmeter measures gravimetrically the total amount of abraded dust, testing of treated seeds is quick and inexpensive, as no analytical chemistry is involved.

The working principle of the Heubach - dustmeter is that coated seeds are mechanically stressed inside a rotating drum, thus simulating mechanical stress which coated seeds routinely experience in commercial practice, e.g. via bagging, transporting, sowing etc.. A vacuum pump creates an air flow through the rotating drum, glass cylinder and the attached filter unit. Through the airflow, abraded dust particles are finally collected on a filter-disc inside the filter unit (Figure 6). While floating dust particles settle on the filter disc, coarse non-floating particles are separated and collected in the glass cylinder. The amount of floating dust finally collected on the filter disc is the so-called Heubach-value (HV), which is generally expressed - in case of treated corn seeds - as g dust/100,000 seeds (the amount of dust can also be expressed related to the weight of seeds, as g dust/100 kg seeds).

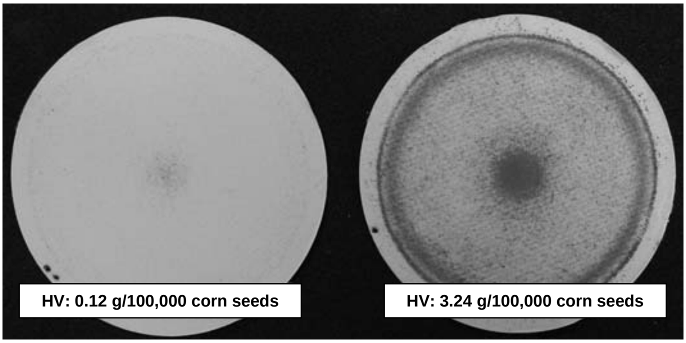


Figure 6 Collected dust deposits on Heubach filter discs

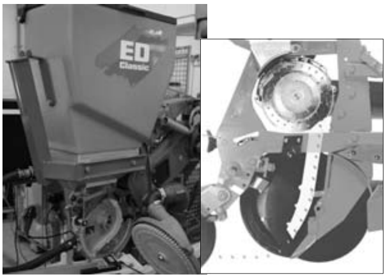
Stewardship measures of bayer cropscience after the bee incident: As an insufficient seed treatment quality has been identified as the main reason for the bee incident in the Upper Rhine Valley in Germany in 2008, Bayer CropScience has initiated an extensive stewardship program for improving seed treatment quality, by

- advising all seed companies which receive insecticidal seed treatment formulations from Bayer CropScience, to implement where necessary, adequate measures to assure maximum cleanliness of the seeds entering the seed treatment process,
- initiating training programs for operators of seed treatment machinery all across Europe, in order to further improve the correct setting of machinery parameters, e.g. mixing time, which may significantly affect the final seed coating quality,
- assisting European seed treatment companies in identifying and choosing adequate adhesives / film-coatings, to achieve a maximum adhesion of the seed treatment products on the seed and to minimize dust abrasion,
- requesting samples of treated seeds from each seed-treatment facility during the start-up period of this stewardship program, before selling insecticidal seed treatment products on commercial scale, in order to verify whether the initiated stewardship measures have been adequately transposed to the actual seed treatment processes
- taking the initiative to provide Heubach - dustmeter equipment to various independent laboratories specialized on seed coating quality investigation in various countries, in order to offer widespread services for Heubach dust measurements to seed treatment companies across Europe (moreover, Bayer CropScience provides Heubach test services at its headquarter and country subsidiaries, where applicable).
- training the laboratory personnel involved in dust measurements to correctly implement Heubach dust abrasion measurements.

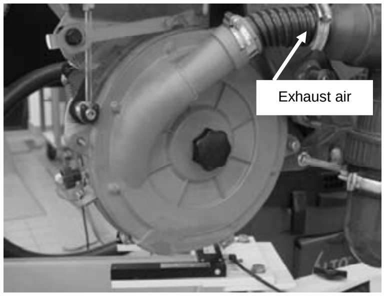
Conclusion on seed treatment quality: Bayer CropScience has initiated extensive stewardship measures all across Europe. The initiative aimed to rise awareness within the seed treatment community to pay particular attention to the abrasion resistance of the final seed coating and to provide expert knowledge and assistance, where required, to assure “Good Seed Treatment Practice”.

Modifications of the drilling machinery: Based on the geographical correlation analysis outlined above, insufficient seed treatment quality in combination with standard vacuum-pneumatic sowing equipment has turned out to be the main reason for the bee incident in the Upper Rhine Valley. Moreover, the geographical correlation analysis further revealed that in cases where low-drift technology was used, bee damages have not been reported, even in case inappropriately treated seeds were sown.

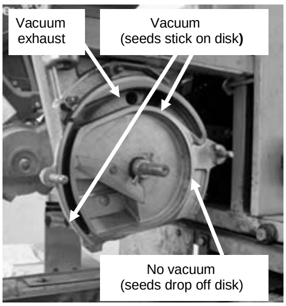
Principle of vacuum-pneumatic corn sowing: Corn is precision-drilled via so called single-kernel sowing devices. To achieve a precise deposition of the seeds in the soil, all vacuum-pneumatic sowing machines (standard and modified) aspirate corn seeds from a deposit via suction pressure, generated by a central fan, on a perforated disk. On the individual perforations of this disk, corn seeds are separated / individualized by sticking to the holes as long as the negative pressure (vacuum) is sustained. Due to the forward movement of the sowing disk, individualised seeds will loose their contact to the vacuum and will therefore finally drop into the furrow, one after the other with a concrete spacing (Figure 7).



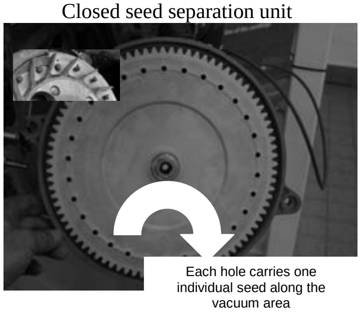
Vacuum-pneumatic sowing device (open seed-separator)



Closed seed separation unit



Vacuum area in separator (without perforated disk)



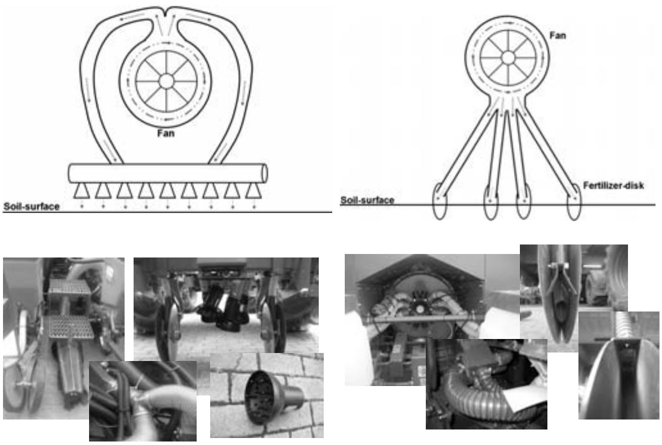
Perforated disk

Figure 7 Principle of pneumatic single-kernel corn sowing

Standard vacuum-pneumatic drilling machines: By spring 2008, standard vacuum-pneumatic sowing devices were state of the art technology for corn sowing in Germany, comprising high market shares. By using this technology, the resulting exhaust air, which contains varying quantities of abraded seed treatment particles (depending on the machinery type and the quality of the corn seed-coating), is emitted with a high flow-off velocity from one single outlet into the air. The air-stream outlet is generally placed directly on the fan, approximately 1.5 - 2 m above the ground. This construction allows for a rather huge dispersion of abraded seed-coating particles in the environment, particularly in the case of an inappropriate seed-coating quality (Figure 8).

Figure 8 Standard vacuum-pneumatic drilling machine with an upward directed air-stream outlet, directly from the fan

Modified vacuum-pneumatic drilling machines: In a co-operative approach, engineers and application specialists of Bayer CropScience and of various manufacturers of vacuum-pneumatic sowing equipment have developed during 2008 concepts of an effective machinery modification, in order to transform existing vacuum-pneumatic drilling machines into low-drift sowing equipment by means of modification kits. Although the developed modification kits differ e.g. in appearance, dimension and exact technical set-up, all modifications follow the same principle approach: the total air-stream generated by the fan to maintain the suction pressure - which was formerly ejected from one single outlet with a high flow-off velocity (see above) - is now divided via several tubes of a rather large cross-sectional area into sub-streams, which are finally released close to the ground. On ground-level, the exhaust air is released via diffusers, cushions or within fertilizer-disks - with or without supporting the fertilizer flow. Overall, the exhaust air is not longer ejected into the environment from approximately 1.5 - 2 m above the ground, but rather gently released close to the soil surface (Figure 9).



Release of exhaust air close to the ground by means of cushions or diffusers

Release of exhaust air close to the ground within fertilizer-disks

Figure 9 Modified vacuum-pneumatic technology

Achievements: Finally, a series of different modified vacuum-pneumatic drilling machines from various manufacturers could be completed right in time to be subject to field testing in summer 2008, in order to investigate whether under field conditions relevant for the commercial corn sowing practice, the developed low-drift technology added to existing vacuum-pneumatic sowing equipment will in fact lead to a significantly reduced off-crop exposure, as intended (for results see: Experimental approach: reality check of the effectiveness of improvements in a drift field trial). Moreover, in addition, independent tests thereafter, the official German Federal authority in charge of approving low-drift technology for both, spray-application and seed-sowing devices (JKI), further examined the five developed modification kits together with other modification kits provided until autumn 2008, for their effectiveness in drift reduction.

Overall, following intensive efforts of the engineers and application specialists of both, machinery manufactures and Bayer CropScience, ≈98% of the European manufactures of vacuum-pneumatic corn sowing machines are now able to provide modification kits for their existing fleet of vacuum-pneumatic sowing equipment. The costs of the modification-kits are generally in the range of several hundred € and the kits can be easily fitted to existing machinery in professional service centres.

Experimental approach: reality check of the effectiveness of improvements in a drift field trial

Study setup and results: In summer 2008, Bayer CropScience conducted an extensive field dust drift study with Poncho Pro®-dressed corn seeds. Overall, more than 70 ha of agricultural land, typical for corn growing under European conditions has been employed for the test program. The machinery under investigation comprised a series of different corn sowing equipments, involving a realistic worst-case unmodified vacuum-pneumatic corn drilling machine as a reference together with five modified vacuum-pneumatic drilling machines of various manufactures. The aim of the modification was to implement low-drift technology to vacuum-pneumatic drilling machines. In addition, also one corn drilling machine which operates with compressed air as well as one mechanical corn sowing machine have been tested. The latter two machines (compressed air and mechanical) were commercially available and not modified, however, supposed to apply low-drift technology due to their specific technical setup. For all investigations, each drilling machinery was tested in the field by sowing dressed corn seeds on an area of approximately 1.0 ha at a drilling rate of 80,000 seeds/ha. In order to investigate and compare the performance of the supposed low-drift drilling technologies in terms of off-crop exposure, the drilling equipment under investigation was uniformly operated with a seed-coating quality, characterized by a measured dust abrasion value of 1.2 g dust/100,000 corn seeds, as determined by the Heubach dust abrasion test. In the following, this particular seed treatment quality is referred to as "HV 1.2 - seeds" (= seeds with a Heubach Value of ≈1.2 g dust/ 100,000 corn seeds). A better seed-treatment quality than HV 1.2 has not been tested with the low-drift drilling technology due to constraints with the analytical quantification of the emitted dust, particularly at greater distances from the drilling area. Moreover, the influence of the seed-coating quality in terms of off-crop exposure has been investigated with the realistic worst-case unmodified vacuum-pneumatic corn drilling machine (= not low-drift). This machine has been operated with two seed-treatment qualities, i.e. with HV 1.2 - seeds and with HV 0.1 - seeds (= seeds, with a Heubach Value of ≈0.1 g dust/100,000 corn seeds).

At various distances adjacent to the drilling area, Petri-dishes and passive dust-drift collectors were installed in the off-crop sampling area during the drilling procedure. Whereas the Petri-dishes were placed on the soil surface to collect the ground-deposable dust fraction ("primary drift"), the passive dust-drift collectors were installed at various heights above the ground to collect the airborne dust fraction ("atmospheric drift"). After drilling was completed, the samples were collected. Moreover, in order to investigate whether the dust that deposited during sowing within the drilling area will be dislodged from the soil surface and transported downwind ("secondary drift"), a further set of Petri-dishes was installed downwind in the off-crop sampling area after the sampling of the Petri-dishes for the primary drift, to collect dust, potentially dislodged from the soil surface. After an exposure period of 24 hours, these Petri-dishes were collected. The samples were processed in the analytical laboratory of Bayer CropScience AG; the content of clothianidin was determined by using High Performance Liquid Chromatography coupled with tandem mass-spectrometry (HPLC-MS/MS). The results of the study are depicted in Figure 10 - 13.

Discussion of the study results: The comparison of different seed-coating qualities (quality in terms of abrasion resistance as measured by the Heubach abrasion test) on an identical, unmodified, vacuum-pneumatic corn drilling machine revealed that seed-coating quality is a major factor which significantly impacts both, ground deposition and atmospheric drift (Figure 10).

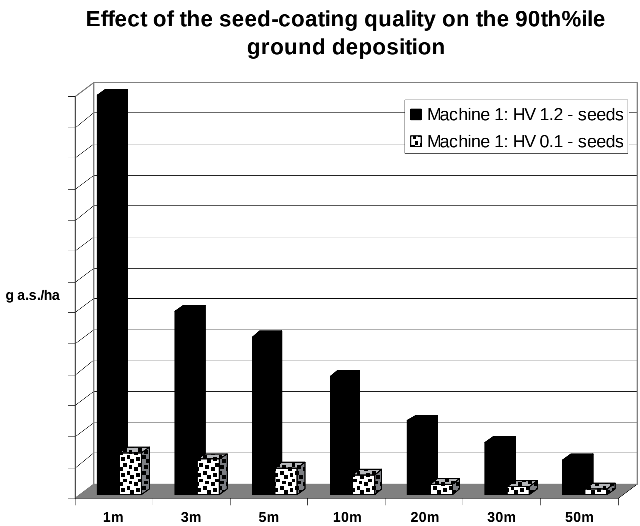


Figure 10 Effect of seed-treatment quality on off-crop ground deposition. Machine 1 is an unmodified reference machine.

The comparison of the corn sowing equipment which was tested with HV 1.2 – seeds, showed that all modified vacuum-pneumatic corn drilling machines along with the mechanical corn drilling machine (i.e. no air assistance) and the corn drilling machine which is operated with compressed air, performed in a comparable way, leading to a significant drift reduction compared to an unmodified vacuum-pneumatic corn drilling machine with an air-stream release directly from the fan (i.e. from one single outlet) (Figure 11).

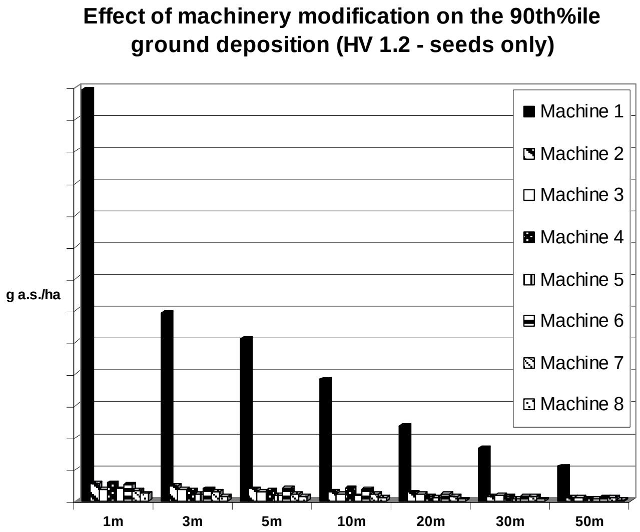


Figure 11 Effect of machinery modification on off-crop ground deposition. Machine 1 is an unmodified reference machine, machines 2-8 are all low-drift machinery

This significant drift reduction became obvious for both, ground deposition and atmospheric drift (Figure 12).

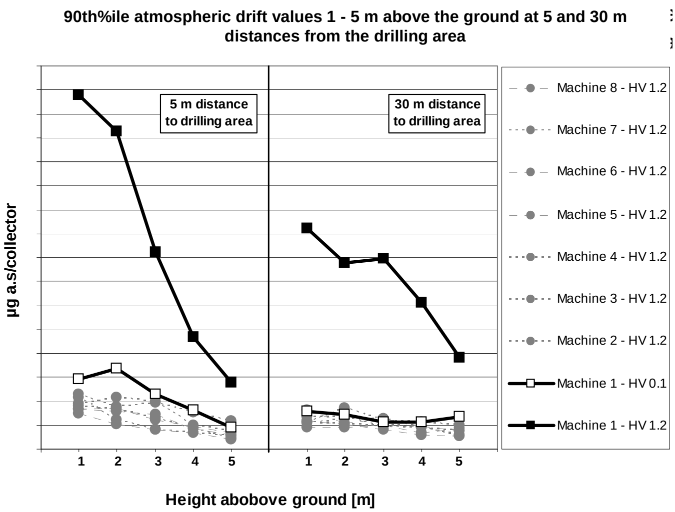


Figure 12 Effect of machinery modification and seed-coating quality on airborne dust. Machine 1 is an unmodified reference machine, machines 2-8 are all low-drift machinery

Although there were variable weather conditions within the 24-hours post-drilling periods during the investigation of the different machinery and seed-coating qualities, the obtained data concerning secondary drift processes show a consistent picture: Secondary drift processes (i.e. the downwind transport of dislodged dust particles deposited during the drilling operation of the soil surface) takes place, if at all, in a negligible extent that it can hardly be detected (Figure 13).

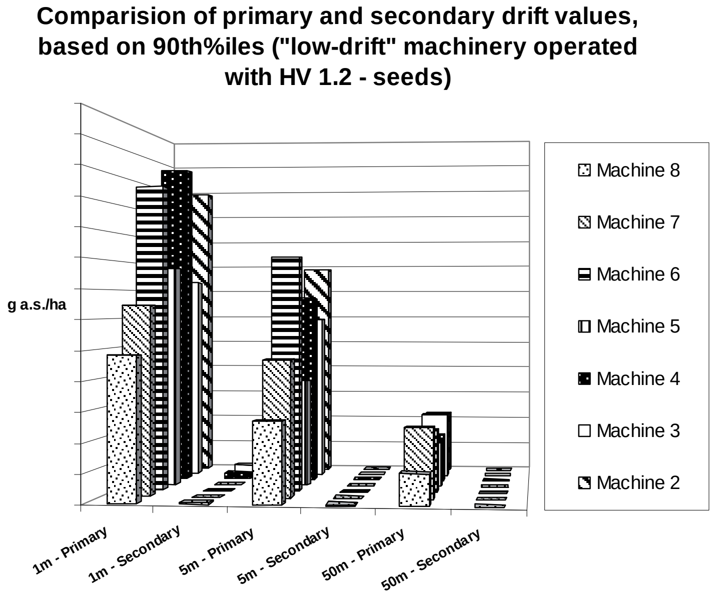


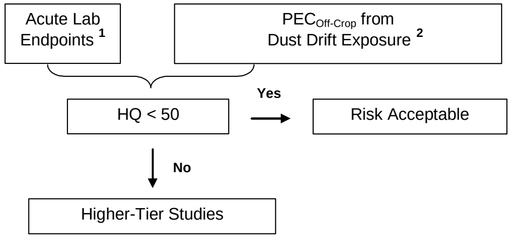
Figure 13 Comparison of primary drift (i.e. dust-drift during and immediately after drilling) and secondary drift (i.e. dust particles dislodged from the drilling area and transported downwind within a 24 h period after end of drilling); machine 2-8: low-drift machinery

<u>Conclusions from the drift field trial</u>: Overall, it could be demonstrated that all modifications mounted to existing vacuum-pneumatic sowing equipment - which all followed the same principal approach (see: Modified vacuum-pneumatic drilling machines) - allow for a successful implementation of low-drift technology, which proved itself to be as effective as e.g. the low-drift technology of a mechanical sowing machine which operated without any air assistance.

Moreover, the effectiveness in terms of a significantly reduced off-crop exposure achieved by the use of the tested modification kits as well as of further modification kits, has been additionally confirmed by independent tests of the competent German Federal authority (JKI). The current status of officially approved low-drift technology for commercial vacuum-pneumatic corn sowing equipment can be found on the webpage of the JKI (http://www.jki.bund.de/); the confirmed drift-reduction amounts to 90% compared to not-modified equipment.

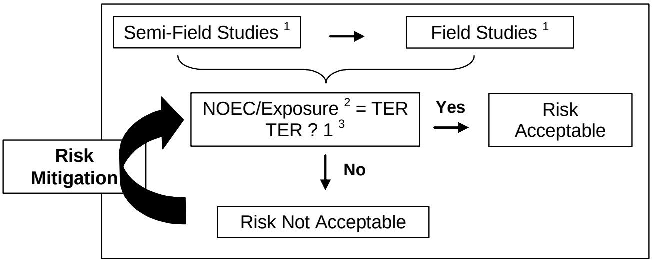
Honey bee risk assessment

<u>IVA Dust risk assessment proposal for bees</u>: The proposed risk assessment scheme outlined in the following was corroborated by an expert working group of the German Industrieverband Agrar (IVA, the association of the crop protection and fertilizer industry in Germany) (Figures 14, 15).



[1] Standard oral and contact acute study results; [2] PEC from off-crop dust exposure including mitigation

Figure 14 Tier 1 honey bee risk assessment scheme for dust exposure



[1] Acute as well as brood effects should be assessed (studies with spray application, potentially studies with dust application); [2] Including refined exposure assessment; [3] TER threshold value depends on available data

Figure 15 Higher-tier honey bee risk assessment for dust exposure

In a Tier 1 screening approach, a hazard quotient is calculated in order to identify those compounds which require higher-tier assessment concerning their risk to honey bees under relevant use conditions. If the hazard quotient, (HQ), based on the lowest toxicity endpoint under standardised laboratory conditions in combination with realistic worst-case exposure to be expected in the off-crop area, is below the conservative threshold value, no further activities are considered necessary. If the HQ is above the trigger value of 50 higher tier studies are required. In the higher tier risk assessment, a TER (Toxicity-Exposure Ratio) approach is applied and exposure data are compared with the results from tunnel or field studies where bees were exposed to the compound under consideration. Tentatively, studies with spray formulations containing the same active compound as the evaluated seed-dressing formulation is considered appropriate for this step of the risk assessment.

Exemplary bee risk assessment for exposure to clothianidin via dust during corn drilling (Bayer CropScience): Following the IVA honey bee risk assessment proposal outlined above, the rate of clothianidin [g a.s./ha] which has not induced increased mortality in a honey bee semi-field cage study, where clothianidin was sprayed into a full-flowering and bee-attractive crop during honey bees were actively foraging (unpublished GLP study data), is compared to the 90th%ile of the field-measured clothianidin exposure values in the off-crop area (ground deposition, 1m distance directly adjacent to the corn drilling area), the following TER value for clothianidin is calculated under consideration of the following parameters:

- a seed loading of 1.25 mg clothianidin a.s./kernel (Poncho Pro®),
- a Heubach dust abrasion value of ≈1.3 g dust / 100,000 corn seeds, and
- modified vacuum-pneumatic corn drilling equipment, mechanical corn drilling equipment or corn drilling equipment which operates with compressed air,

Comparing the toxicity and the exposure value as outlined above, the resulting TER-value is 6. From this TER figure, it can be concluded that it is unlikely that there is an unacceptable risk for honey bees from abraded clothianidin deposits associated with the aforementioned seed-coating quality and machinery parameters. The margin of safety can be further improved by an enhanced seed-coating quality.

Final conclusions and outlook

Substantial work has been undertaken to investigate the causal factors that constituted the bee incident in the Upper Rhine Valley in 2008. Intensive activities were dedicated to develop optimizations in the areas which were identified as key factors for appropriate risk mitigation for seed treatments, i.e. seed-dressing quality and drilling technology. It was demonstrated in comprehensive field studies under realistic conditions that the developed mitigations measures work efficiently.

Therefore it can be concluded that by implementation of the outlined optimizations, the exposure of bees to dusts from seed-coating products during the drilling process can be minimized by orders of magnitude, and that a bee-safe use of insecticidal seed-dressing products can be ensured.

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Spring honey bee losses in Italy

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Abstract

Background: During last years several cases of bee losses have been reported during the period of corn sowing in different European countries. In Italy an institutional system for bee losses survey does not exist and therefore some Italian regions decided to organise an official network to collect data and analyse dead bee samples.

Results: Collected data indicate that the higher number of bee losses events occurred in intensively cultivated flat areas, located in the North of Italy, mainly during or after corn sowing. The chemical analyses of dead bees revealed the presence of three neonicotinoid residues: imidacloprid was found in 25.7% of the sample,