

Background

In de Tweede Kamer is op 17 februari 2011 motie 19 aangenomen. Deze motie betreft de herbeoordeling van bestrijdingsmiddelen op basis van neonicotinoïden voor het onderdeel (subletale) effecten op bijen. Dit document bevat de beoordeling van het risico voor bijen van momenteel in Nederland toegelaten middelen op basis van clothianidin. Deze middelen zijn in onderstaande tabel weergegeven.

Gewasbeschermingsmiddelen op basis van clothianidin

toelatingnr	middelnaam	toelatinghouder	werkzame stoffen	toepassing	formulering	Toepassing(en)
13044	PONCHO BETA	Bayer CropScience B.V.	clothianidine 400G/L # beta-cyfluthrin 53,3G/L	Professioneel	Suspensie concentraat voor zaadbehandeling	zaadcoating in bieten
13276	PONCHO ROOD	Bayer CropScience B.V.	clothianidine 600G/L	Professioneel	Suspensie concentraat voor zaadbehandeling	zaadcoating in snij- en korrelmaïs

Er zijn geen biociden en geen niet-professionele middelen toegelaten op basis van clothianidin.

A. Plant protection products

Risk assessment is done in accordance with Chapter 2 of the RGB published in the Government Gazette (Staatscourant) 188 of 28 September 2007, including the update of 20 October 2009, which came into effect on 1 January 2010. The bee risk assessment is based on the most recent guidance document, which is EPPO 2010. This includes methodology to assess the risk from systemic substances.

Clothianidin is placed on Annex I of 91/414/EEG since 08/01/2006 (2006/41/EC). In Commission Directive 2010/21/EU, the Inclusion Directive of clothianidin was amended with additional provisions to avoid accidents with seed treatments. The provisions relevant for honeybees are now as follows:

"PART A

Only uses as insecticide may be authorised.

For the protection of non-target organisms, in particular honey bees, for use as seed treatment:

- the seed coating shall only be performed in professional seed treatment facilities. Those facilities must apply the best available techniques in order to ensure that the release of dust during application to the seed, storage, and transport can be minimised,
- adequate seed drilling equipment shall be used to ensure a high degree of incorporation in soil, minimisation of spillage and minimisation of dust emission.

Member States shall ensure that:

- the label of the treated seed includes the indication that the seeds were treated with clothianidin and sets out the risk mitigation measures provided for in the authorisation,
- the conditions of the authorisation, in particular for spray applications, include, where appropriate, risk mitigation measures to protect honey bees,
- monitoring programmes are initiated to verify the real exposure of honey bees to clothianidin in areas extensively used by bees for foraging or by beekeepers, where and as appropriate.";

The risk assessment is based on the final LoEP of October 2005 and additional data from the applicant (presented in Annex I). Also, information from the public literature is taken into account (presented in Annex II).

A.1 Professional uses of plant protection products: seed treatments

toelatingnr	middelnaam	toelatinghouder	werkzame stoffen	dosering	formulering	Toepassing(en)
13044	PONCHO BETA	Bayer CropScience B.V.	clothianidine 400G/L # beta-cyfluthrin 53,3G/L	1x 60 g a.s./ha (clothianidin)	Suspensie concentraat voor zaadbehandeling	zaadcoating in bieten
13276	PONCHO ROOD	Bayer CropScience B.V.	clothianidine 600G/L	1x 50 g a.s./ha	Suspensie concentraat voor zaadbehandeling	zaadcoating in snij- en korrelmaïs

Risk assessment for bees

Exposure to honeybees may occur via several routes, which will be discussed separately below.

Direct exposure

Direct exposure to clothianidin should be avoided because of its high acute toxicity to bees: LD₅₀ = 0.00379 and 0.04426 µg a.s./bee (oral and contact, respectively) according to the LoE. It is noted that on May 26th 2011, the applicant submitted a new acute toxicity study to honeybees which showed slightly lower values: 0.0025 and 0.0389 µg a.s./bee for oral and contact toxicity, respectively. This new study (Schmitzer 2008) was submitted too late for full evaluation and is not essential for the risk assessment as the EU-agreed endpoint is in the same range (indicating the high toxicity of the active substance). Therefore, the new study is not included in the LoE and the current risk assessment.

1) *In-field*

In-field exposure during sowing is not expected for the proposed uses, because they are seed treatment uses.

2) *Off-field - dust from treated seed*

Dust drift from seed is a relevant exposure route for the use in beets and maize, because the seeds are sown outside. The risk that dust from the seed coating reaches neighbouring crops or other flowering plants and in that way exposes bees to the a.s., depends on the type of coating in combination with the type of sowing. This assessment is based on the dust drift matrix available at www.ctgb.nl.

Sowing of beets is done mechanically and seeds have a film coating. No dust drift is expected. The recently submitted studies on dust drift from sugar beet sowing (see LoE, section *dust deposition sugarbeet*) confirm this expectation. The risk via this route is acceptable for the use in beets without additional measures.

Maize seeds are coated with a normal/basic coating, so dust formation cannot be excluded. Whether this dust can be expelled outside the field depends on the type of machinery. The sowing of maize is done with pneumatic machines. The pneumatic machines used for maize sowing have been adapted since 01/2010 to ensure that the air flow is sent downwards, towards the maize field and not upwards. Furthermore, the dust level of maize seeds is kept to

a minimum and sowing is not done under windy weather conditions.

The worst case value for drift outside the field when maize sowing is done according to these restrictions is 0.55% of applied dose (at 5 m from the field, see LoE section *dust deposition maize*). This leads to an off-field dose of 0.55% of 50 = 0.28 g a.s./ha.

The NOER for mortality of dust exposure to clothianidin was set at <0.50 g a.s./ha in a cage study (Bakker 2010, LoE section *dust toxicity*). Thus, for the use in maize mortality of honeybees which are exposed to dust from sowing cannot be excluded based on this value and further work (e.g. higher tier studies) is necessary. However, this cage study also showed that mortality returned to normal levels immediately after moving the hives to an uncontaminated area and that no effects of the treatments on colony condition were observed.

Effects from exposure to dust from maize sowing were also studied in a field test (Garrido 2010, LoE section *dust toxicity*). In this study, the application rate was higher than proposed for Poncho Rood (132.9 vs. 50 g a.s./ha). As explained in the study summary in the LoE, it is expected that no adverse long term effects are induced by exposure to low levels of abraded dust from clothianidine dressed maize seeds, released during sowing. This study is still running and the final report, scheduled for summer 2011, will include an assessment of the overwintering performance of the colonies.

In addition to the semi-field and field studies, long-term monitoring studies were performed which studied the effects on honeybee colonies after exposure to dust from maize sowing.

In Germany, monitoring was set up after the dust drift incident with clothianidin-treated maize in 2008 (Liebig 2008 and 2009, LoE section *bee monitoring*). The study authors concluded that even if exposure of dust during sowing of maize seeds has adverse effects on bee colonies, they can recover and successfully overwinter. This conclusion is probably correct, but the evaluator noted some shortcomings which should be addressed by the applicant (see below).

In four studies on isolated field plots in France, honey bee colonies were exposed during sowing of maize seeds, seed-treated with clothianidin at a rate of nominally 0.5 mg clothianidin a.s./kernel (50 g a.s./ha) and during the subsequent period when guttation was regularly observed on the maize seedlings (Liepold, 2010 a-d, see LoE section *guttation and dust exposure*). The study author concluded that in none of the studies adverse effects on honey bees and honey bee colonies have been observed, neither during the sowing operation of the clothianidin-treated maize seeds nor during the subsequent growing period of the maize seedlings when guttation occurred. The evaluator accepted this conclusion for guttation and also accepted the conclusion that maize seed drilling (and subsequent guttation of seedlings) had no short term effects on bee colony development during the exposure period (24 days) for one of the four studies (Champagne area). In the other three areas, it was considered not likely that exposure to dust during and shortly after drilling has had considerable effects on bee mortality or colony development during the exposure phase, but there were some shortcomings in the analysis of the data and re-analyses of the data is considered necessary before proper conclusions can be drawn from these three studies.

The study evaluator remarked that interpretation of most of the long-term studies in France and Germany would be improved by statistical hypothesis testing (e.g. a pre-post repeated measures design). However, statistical analysis of bee field studies is not commonly performed in the current European risk assessment framework; instead, the results are interpreted by expert judgment. Based on expert judgment (of Ctgb and the study evaluator) of the wealth of available data for clothianidin, it is concluded that the long-term effects on honeybee colonies after exposure to dust from maize sowing are acceptable. It is therefore concluded that the risk

from dust drift of Poncho Rood is acceptable, provided that the level of dust drift is kept to a minimum.

To ensure this, and reduce exposure outside the field where flowering plants may be present as much as possible, the dust level of maize seeds should be as low as possible, deflectors should be used and sowing should not be done under windy weather conditions. Incidents with maize sowing causing acute mortality of bees foraging on neighbouring areas (in 2008 in Germany, Slovenia and Italy; probably also in 2011 in Slovenia, this incident is still under investigation) show that it is very important that these conditions are met. In the Netherlands, increased bee mortality after maize sowing has never been reported so far.

The following restrictions should be mentioned on the product label for maize (already prescribed since January 2010):

Behandeld zaad mag bij het opzakken geen hoger stofgehalte hebben dan 0,75 g stof per 100.000 zaden (volgens de Heubach-methode).

Om de bijen te beschermen moet blootstelling via stofdrift geminimaliseerd worden. Om dit te bereiken dienen bij het uitzaaien van het behandelde zaad specifieke instructies gevolgd te worden die vermeld staan op de zakken behandeld zaad.

Het volgende moet worden vermeld op de zakken met behandeld zaad:

'Voor het zaaien

Breng bij het vullen het eventueel aanwezige stof onderin de zaaizaadzak niet over in de zaaimachine.

Bij het zaaien

Zaai geen behandeld zaad bij sterke wind en zaai de aanbevolen hoeveelheid zaaizaad. Wanneer een pneumatische zaaimachine wordt gebruikt, moet de luchtstroom met eventueel daarin aanwezig stof van behandeld zaad naar het grondoppervlak of in de grond worden gericht via zogenaamde deflectoren.'

Thus, with these restrictions and based on expert judgment of the wealth of available data for clothianidin, it is concluded that the long-term effects on honeybee colonies after exposure to dust from maize sowing are acceptable.

Indirect exposure via systemic working mechanism

Due to its systemic nature, the a.s. can be taken up by plants. If this plant carries flowers, bees may be exposed to clothianidin or its metabolites via nectar and/or pollen. This route may be relevant for the crop itself, weeds and succeeding crops. Guttation droplets may contain the active substance and/or metabolites. Also, the risk via honeydew from aphids must be assessed.

The risk of the metabolites is considered to be covered by that of the parent, as the metabolites are less or much less toxic than the parent (metabolite TZNG is of moderate toxicity to bees: acute LD₅₀ = 3.9 µg TZNG/bee; all other metabolites are not toxic to bees, as indicated in the DAR) and in all residue studies the analysed concentrations of TZMU and TZNG in nectar and pollen were below the limit of detection of 0.3 µg/kg. Therefore, only the parent substance clothianidin is considered.

The EPPO scheme (2010) indicates that when risks from systemic substances can be expected based on acute toxicity of the substance, toxicity after longer-term exposure should

be considered. For clothianidin, a chronic (10-d) toxicity study with adult bees was done. The 10-d NOEL was 10 µg/L and since the density of the sucrose solution was ca. 1.18 g/cm³, this corresponds to a value of 8.5 µg/kg. The study author calculated that at this concentration, the cumulative dose over 10 days was 3.8 ng/bee.

Furthermore, a larvae toxicity study indicates that the NOEC for adverse effects on larvae development should be set at 20 µg a.s./kg diet.

Based on these two laboratory studies, the level at which no adverse effects are expected from clothianidin is 8.5 µg/kg.

The applicant also submitted two further laboratory studies (Simoens & Jacobs 2005 a+b). One study aimed at demonstrating whether clothianidin, brought into the bee hive via contaminated pollen, will be transferred to the next generation of honey bees (larvae) via larval food (royal jelly) provided by nurse bees. In the other study, the effect of clothianidin in bee bread (mixture of pollen and honey consumed by nurse bees to produce royal jelly) on food gland development has been determined. According to the applicant, the residue analysis of all royal jelly samples revealed that there is no transfer of clothianidin from the food of nurse bees (bee bread contaminated with 10 µg/kg of clothianidin) to the royal jelly provided to the larvae when the nurse bees are exclusively fed with clothianidin-contaminated bee bread. Moreover, no impact on the development of the brood food glands of nurse bees was found when nurse bees exclusively fed on bee bread, contaminated with 10 µg/kg of clothianidin. These studies were submitted too late (May 26th 2011) to enable a full evaluation. Based on the summary of the applicant, they do not give concerns about the relevant long-term laboratory endpoint, which is now determined for adult bee mortality at 8.5 ppb and for larval development at 20 ppb. The studies are also not required according to the risk assessment scheme. For these reasons, they are not included in the LoE and the current risk assessment but will be evaluated for future risk assessment of clothianidin.

1) Residues in nectar and pollen of the treated crops

Beets are not supposed to flower during cultivation. Therefore, no exposure via nectar or pollen from the treated crop itself will take place.

Maize, however, will flower and bees can collect the pollen from maize. Therefore, the exposure route via maize pollen must be considered.

Several residue studies in maize are available in the DAR (LoE, section *Field or semi-field studies - residue studies*) in which the seeds were treated at the intended use rate. In none of these studies residues of the metabolites TZMU or TZNG in pollen was found (residue usually <0.3 µg a.s./kg (LOD), once < 1 µg a.s./kg (LOQ)). The retrieved residues of clothianidin in maize pollen ranged from 2.1-6.2 µg a.s./kg pollen (mean value not calculated; from here on, 'ppb' will be used instead of µg a.s./kg).

The applicant now submitted an additional residue study in maize (LoE, section *Residues*).

Residue measurements in maize pollen at a worst case dose rate (1.25 mg a.s./seed, as opposed to the proposed 0.5 mg a.s./seed) showed that the mean values of clothianidin in pollen are 3.4 ppb in pollen from plants and 1.1 ppb in pollen taken from bees. Only very exceptionally is a value above the NOEC of 8.5 ppb found.

The risk to adult bees foraging on maize pollen can be estimated by using the data from Rortais et al. (2005), as indicated in EPPO 2010. Nurse bees are expected to consume the highest amount of pollen of all categories of bees: 65 mg/bee in 10 days.

For the chronic risk assessment, mean residue values are appropriate (see EPPO 2010, note 10). The mean residue value in pollen of 3.4 µg/kg (taken from the study with worst-case seed treatment rate of 1.25 mg a.s./seed) leads to a possible intake of clothianidin by nurse bees of (65 mg*3.4 µg/mg=) 0.221 ng/bee in 10 days. This value can be compared to the chronic NOEL

for adult bees of 3.8 ng/bee in 10 days, which leads to a TER of 17, indicating a low risk. This calculation assumes that all pollen is taken from maize, which may be considered a worst case. E.g. the French Authority uses a maximum rate of 80% maize pollen in pollen intake based on an INRA survey on the collection of maize pollen by forager bees (information from the French risk assessment of Cruiser 350 dd. December 2009).

Furthermore, since the residues in maize pollen are always below the NOEC for larvae development of 20 ppb, even at a seed treatment rate which is more than twice as high as the proposed rate, the risk to larvae fed with pollen from treated maize is expected to be low.

Thus, based on the residue levels in pollen and the laboratory endpoints for chronic adult mortality and larvae development, the risk to honeybees from foraging on maize which has been treated with clothianidin is expected to be low. This expectation can be checked by looking at the higher tier studies.

In the DAR of clothianidin, several higher tier studies are presented (see LoE, section *Field or semi-field tests*). Semi-field and field studies are available in which colonies were foraging on seed-treated flowering crops (sunflower and oilseed rape) or fed with treated pollen or honey. No adverse effects on bees were found. However, in all studies in the DAR, the observation period was short, only up to a couple of weeks. This means that less than one full brood cycle was covered during these trials. It was concluded in the DAR that the risk to bees from the uses in maize (53.8 g a.s./ha) and sugar beet (78 g a.s./ha) is acceptable based on the higher tier studies in the DAR. These uses in principle cover the proposed uses in the Netherlands. However, only the risk of exposure via the crop itself was considered and effects on bees were not studied for longer than two weeks. Ctgb considers that the risk of this persistent, systemic substance should be more thoroughly investigated than was done in the DAR.

Studies of longer duration are now available to assess the possible long-term effects of exposure to clothianidin in maize pollen. In France, on three locations long-term exposure studies on honey bees are running (Hecht-Rost and others, LoE section *long-term studies after exposure to flowering maize*), which include overwintering success. In these studies the same honey colonies are exposed for three years in succession to flowering maize on isolated field plots, which have been grown from maize seeds, seed-treated with clothianidin at a rate of nominally 0.5 mg clothianidin a.s./kernel (nominal dose rate: 50 g a.s./ha; no exposure during sowing, only from foraging on pollen). Exposure lasts for 10-12 days per year, the period of flowering, after which the colonies are transferred to a monitoring site. Residues in maize pollen are low (reported as 1, 3 and 5 µg/kg in the three studies).

The study authors conclude that on the basis of available data in the interim reports, no long-term adverse effects on honey bees and honey bee colonies are induced from exposure to flowering maize grown from clothianidin dressed maize seeds. As indicated in the LoE, one of the three studies was not accepted in its current state (re-analysis of the data is considered necessary). The other two studies however indicate that there are no long term effects induced by exposure to flowering maize grown from clothianidin dressed maize seeds.

The evaluator remarked that interpretation of these long-term studies would be improved by statistical hypothesis testing. However, statistical analysis of bee field studies is not commonly performed in the current European risk assessment framework; instead, the results are interpreted by expert judgment.

Based on the above risk assessment using laboratory data, higher tier studies including multi-year trials, and measured residue data, long-term adverse effects on honeybee colonies when exposed to flowering maize treated with Poncho Rood at the proposed rate are not expected, so risk is acceptable.!

2) Flowering weeds

In the proposed crops, flowering weeds may occur in the field, but exposure via this route is not expected to be very high since a large amount of flowering weeds in fields is adverse to profitable agriculture. Therefore, exposure via this route is expected to be low.

3) Succeeding crops

Based on the persistent nature of clothianidin, it may occur in succeeding crops. This route was not assessed in the DAR. To assess the risk from exposure via succeeding crops, the higher tier studies with flowering crops (sunflower and oilseed rape) available in the DAR will be considered. In these studies, seeds were directly treated, so they may be seen as worst case for exposure from untreated succeeding crops.

Several studies were done with oilseed rape. During the cage tests, treated rape seed was tested in Sweden, France and Great Britain at the intended use rate. In all these studies no increased mortality or behavioral impacts were observed. Residue analyses were conducted which resulted in maximum 8.6 µg a.s./kg rape nectar sampled by bees and maximum 1.7 µg a.s./kg rape pollen sampled by bees. In addition there is a field study (in Ontario and Minnesota) with an application rate slightly lower than the intended use for oil seed rape. No treatment related effects were observed during this field study. Also two tunnel tests are available, established in summer rape fields in Germany at the intended use seed dressing rate. In both studies no treatment related effect on behavior and mortality could be observed. Analyzed residues : maximum 5.4 µg a.s./kg rape nectar and max. 2.5 µg a.s./kg rape pollen. A study on the residue levels of clothianidin and its relevant metabolites in nectar and pollen of winter rape was conducted. In this study the seeds were treated slightly below the intended use rate. The nectar and pollen contained no residues of clothianidin (below the LOD of 0.3 µg /kg), nor of its metabolites TZMU and TZNG.

Studies were also done with sunflower: There are two tunnel tests available established in sunflower fields in Germany, slightly above the intended use rate. During both tests no effects on behavior were observed. On day 3, high mortality was observed in one test. This was probably due to an escape trial of a bee swarm. This high mortality on day 3 is not regarded as treatment related. Analyzed residues : maximum 3.1 µg a.s./kg sunflower pollen and < 0.3 µg a.s./kg sunflower nectar (below the limit of detection).

A test was conducted to investigate the effect of treated sunflower honey on the development of small bee colonies. During the test the behavior was not affected. The honey deposition area and the proportion of the comb area occupied by adults were not affected by the treatment. There was no treatment related effect observed on population growth, queen egg laying activity, larval and pupae abundance. The number of dead bees found at the tent edges in the treatment group was slightly higher than in the control but no dose relationship was observed. On day 22 an abnormal high number of dead bees was found in front of the beehive of the 10 µg/kg group. This effect was not dose-response related and arrived just on one single day. Besides these crop dependent studies two more studies were performed. A field study investigated the effects of treated sugar solution. No treatment related effects on the behaviour could be observed. A raised mortality was observed for the highest test concentration. Therefore the NOEC of this study is established at 20 µg/kg sugar solution. The second test investigated the effects of treated pollen on the development of small bee colonies. The behavior was not affected. The honey deposition area and the proportion of the comb area occupied by adults were not affected by the treatment. There was no treatment related effect observed on the population growth, queen egg laying activity, larval and pupae abundance at 20 µg/kg. No effect on mortality was observed.

The available higher tier trials show that no short-term adverse effects are expected on honeybee colonies when exposed to flowering oilseed rape and sunflower at 50 and 25 g a.s./ha, respectively. It is considered that these studies are relevant to assess the risk of succeeding bee-attractive crops for the proposed uses, even if the use in beets has a slightly higher dose rate. As already noted above, the studies in the DAR are of short duration and do

not assess possible long-term effects on bees. They are however useful for their residue measurements. Using the residue measurements, the risk to bees foraging on pollen or nectar can be estimated by using the data on daily intake from Rortais et al. (2005), as indicated in EPPO 2010. For the chronic risk assessment, mean residue values are appropriate (see EPPO 2010, note 10).

The worst-case mean residue value in pollen of 3.4 µg/kg (taken from the study with worst-case seed treatment rate of 1.25 mg a.s./seed) is used here (it is noted that this is higher than all residues measured in sunflower and OSR pollen). As already calculated above, at this level a low risk is indicated to bees feeding on pollen. Also, the residues in nectar and pollen of directly treated crops are always below the NOEC for larvae development of 20 ppb. Therefore, the risk to larvae fed with forage from succeeding crops is expected to be low.

Considering nectar, nectar foragers are expected to consume the highest amount of nectar of all categories of bees: 224-899 mg sugar/bee in 7 days, which translates into a level of 32 – 128 mg sugar/bee/day. How much nectar or honey intake is needed to reach this sugar intake, depends on the crop and environmental conditions. Rortais et al. give the example of sunflower: when a honeybee requires 1 mg of sugar, it will have to consume either 2.5 mg of fresh sunflower nectar or 1.25 mg of sunflower honey. Thus, a bee would need 80-321 mg sunflower nectar/day or 40-160 mg sunflower honey/day.

Measured residues in sunflower nectar are <0.3 µg/kg (for honey, no measurements are available). The exposure can be calculated as <0.3 ng/g * 0.321 g/bee/day = <0.0963 ng/bee/day. This value compared to the chronic NOEL for adult bees of 0.38 ng/bee/day (calculated from 3.8 ng/bee in 10 days) leads to a TER of >4. Thus, the expected exposure from foraging on treated sunflower is lower than the NOEL for adults (it is noted that the EPPO scheme does not give a trigger value to compare the TER with). This calculation is based on the worst case intake level for nectar foragers and on a <-value for nectar concentration.

Residue levels in rape nectar indicate a higher level: a maximum level of 8.6 µg/kg was found. Sugar content in rape nectar and rape honey is unknown, but assuming comparable content as sunflower, it is clear that with these levels the TER may be above 1. However, it is also clear that it is a very worst case approach to use measured residue values from directly treated crops for untreated succeeding crops.

Therefore, the applicant has now submitted studies (Neuman *et al.* 2005a,b,c; Przygoda *et al.* 2007a,b) in which the residue levels in nectar and pollen of succeeding crops collected by bees have been investigated.

Three scenarios were tested, all taking into account a realistic worst-case long-term soil background concentration after successive use of clothianidin as maize seed treatment over several years:

- 1) Treated and untreated maize, sown in treated or untreated soil (simulating maize mono-culture scenario). Results: The analytically verified soil concentration of 18.0 and 19.2 µg clothianidin/kg soil (day of drilling) did not result in a quantifiable up-take and translocation of clothianidin into pollen of undressed maize (<LOQ of 1 ppb). Clothianidin residues in pollen from clothianidin-dressed maize in clothianidin-pre-treated soil were nearly identical (difference 0.1 µg a.s./kg) to the residues in maize pollen from clothianidin-dressed maize in native soil.
- 2) Untreated OSR sown in treated soil simulating crop failure situation. Results: An analytically verified soil concentration of 21.0 µg clothianidin/kg soil (day of drilling) resulted on average in a residue level of 3.5 µg clothianidin/kg pollen (max. 4.0 µg clothianidin/kg pollen). The induced residue level in nectar was found to be 2.2 µg clothianidin/kg.
- 3) Untreated OSR sown in treated soil simulating realistic succeeding crop scenario. Results: Under still conservative, but more realistic use conditions (long-term background concentration applied via Clothianidin FS 250 spray application on bare soil, followed by immediate incorporation into the soil and by the additional sowing of clothianidin-dressed winter barley at a rate of 80 g clothianidin/ha before sowing of clothianidin-untreated winter-OSR), the maximum residue of clothianidin in bee relevant matrices of a flowering and bee attractive succeeding

crop (nectar and pollen of winter-OSR) was found to be 1.0 µg a.s./kg; all other residue values of clothianidin in the treatment groups were either < LOQ (1 ppb) or < LOD (0.3 ppb); residues of metabolites of clothianidin (TZMU and TZNG) in nectar & pollen in the treatment groups were always < LOD (0.3 ppb).

These studies were submitted on May 26th 2011, which is too late to enable a full evaluation. Therefore, they have not been included in the LoE yet. However, the concentrations used are relevant for the uses proposed in the Netherlands and the studies seem to be well performed and comparable to similar studies with imidacloprid, which have been evaluated. Therefore, they give sufficient confidence to conclude that the residues in succeeding crops will be below the NOEC values for adult bee mortality (8.4 ppb) and larvae development (20 ppb). Therefore, the risk to honeybees from bee-attractive succeeding crops is acceptable. The studies will be evaluated fully by CTgb before the eind of juni 2011.

4) Guttation

Several studies are available in which the risk via guttation from clothianidin-seed-treated crops was considered.

The occurrence of guttation was recorded in twelve commercial sugar beet fields and its adjacent crops or habitats, in a typical German sugar beet growing area. Guttation was observed, but not often (see LoE, section *guttation in sugarbeet*).

In maize, guttation is a much more common phenomenon, which was shown in four trials in France (see LoE, section *guttation and dust exposure in maize*, Liepold 2010 studies). In these trials, seedlings were inspected for guttation droplets from emergence till the occurrence of guttation had stopped for more than five days (24-53 days), several times per day from early in the morning until guttation had stopped for that day (between 11 and 13 h). Bee hives were present close to these fields. Guttation was observed to take place in the morning on the majority of observation days, and timing during the day partly overlapped with the period of high flight activity of the bees. Bees were never observed to collect guttation fluid, and seldom were they seen in contact with guttating plants.

A similar trial was performed in Austria (see LoE, section *guttation and dust exposure in maize*, Lueckmann *et al.* 2010): maize seedlings sown from treated seed were observed for guttation and for bees drinking from guttation droplets. Residues in guttation droplets were measured. This study demonstrated that honey bees do occasionally use guttation fluid as drinking supply, and guttation does contain considerable amounts of clothianidin, diminishing over time, but guttation is not a favoured water source, and mortality of adult bees measured at the hives was generally low, confirming that potential exposure to and/or optake of contaminated guttation fluid did not lead to noticeable increases of adult bee mortality measured at the hive.

These studies sufficiently demonstrated that exposure to and consumption of guttation fluid by foraging bees is unlikely to happen, or only at a very low rate.

Furthermore, due to dangers (e.g. presence of predators) bees are not keen on foraging on plants unless there is a considerable reward (pollen, nectar). Therefore, drinking droplets from plants is not likely to occur in the field (personal communication from a professional beekeeper). Therefore, the risk of guttation is acceptable.

5) Honeydew

According to the new EPPO scheme (2010), exposure to contaminated honeydew is not considered relevant in the case of soil and seed treatments, unless the compound is highly selective towards non-aphid insects (see note 4 EPPO 2010 scheme). Section B.6.1.6 of the DAR of clothianidin describes the effects achieved and the mode of action on target organisms. It is concluded there that "the biological efficacy of clothianidin against the sucking pests tested is roughly comparable to that of imidacloprid and thiacloprid". Based on this, the applicant states: "*As such, when comparing the above mentioned data on clothianidin and imidacloprid, it can be concluded that clothianidin exhibits a similar intrinsic affinity to nAChR-binding sites than imidacloprid at a similar to even higher efficacy against aphids. Thus it can be concluded*

that the sensitivity difference between pest aphids and honey bees is even larger for clothianidin as it is for imidacloprid.”

Based on the above, Ctgb agrees that the risk via honeydew from the proposed uses of clothianidin is acceptable.

Public literature:

The above risk assessment, based on protected data from the applicant, indicates that the risks of the proposed uses of clothianidin are expected to be acceptable, provided that restrictions are mentioned on the labels. In this section it will be considered whether studies available in the public literature domain confirm or contradict this risk assessment. This assessment is based on the preliminary results of a public literature survey which is presented in Annex II.

Bailey *et al.* (2005) confirm the high acute toxicity of clothianidin.

Girolami *et al.* (2009) showed that high residue levels can occur in guttation droplets from maize plants grown from treated seeds. However, the field studies in the industry dossier show that the risk to honeybees from actual foraging on these droplets is very low.

In a field study, Cutler and Scott-Dupree (2007) found no effects on brood and colony development (including overwintering) after foraging on treated oilseed rape (residue levels up to 2.59 ppb, in pollen). This study does not contradict the expectation of low risk from exposure to clothianidin.

Wu (2011) measured clothianidin in brood combs in the USA. The substance was found in 1 of the 13 samples, at a level of 35 ppb. The combs were contaminated with many other substances. Most frequently detected were a number of miticides used by beekeepers against *Varroa*. Delayed development was observed in bees reared in contaminated combs in a cage set-up. However, it is difficult to correlate this effect specifically to clothianidin because combs were contaminated with a cocktail of substances and may have contained also more pathogens than control combs, and because no information is available on how clothianidin contamination could have occurred (relation with agricultural use is therefore unclear). Also, this study does not include the implications for colony survival in the longer term. Therefore, this study does not contradict the above risk assessment.

Furthermore, several large-scale monitoring studies were published in which pesticide residues in bee hives were measured.

In a broad survey of pesticide residues, which was conducted on samples from migratory and other beekeepers across 23 USA states, one Canadian province and several agricultural cropping systems during the 2007–08 growing seasons, Mullin *et al.* (2010) found no clothianidin (although it was included in the analysis). However, they did find 98 other pesticides and metabolites in mixtures up to 214 ppm in bee pollen alone, which according to them represents a remarkably high level for toxicants in the brood and adult food of this primary pollinator. They conclude that the effects of these materials in combinations and their direct association with CCD or declining bee health remains to be determined.

In a large study in Germany (Genersch *et al.*, 2010), many pesticides (including miticides) were found in honeybee colonies. Clothianidin was not detected but it is unclear if it was included in the analysis. In this study, factors which significantly influenced overwintering success were 1) high *Varroa* infestation level; 2) infection with deformed wing virus (DWW) and acute bee paralysis virus (ABPV) in autumn; 3) queen age; 4) weakness of the colonies in autumn. No effects could be observed for *Nosema* spp. or pesticides. The authors however consider that further investigations and controlled experiments are necessary to clarify the relation between pesticides and honeybee colony health in the long-term.

In a study in France (Chauzat et al, 2009), honeybee colony health was studied in relation to pesticide residues found in colonies. Clothianidin was not included in the analysis but other substances were found. No significant relationship was found between the presence of pesticide residues and the abundance of brood and adults, nor between colony mortality and pesticide residues. The authors conclude that more work is needed to determine the role these residues play in affecting colony health.

The (clothianidin and other) residues reported in these publications (except for Cutler *et al.*) cannot be linked to a certain (type of) use. Thus, from the public literature the only conclusion that can be drawn with certainty is that clothianidin is sometimes found in different bee matrices in the field. In these matrices usually a mixture is present of many pesticidal substances. So far, no statistical correlation has been found between the presence of pesticide residues in colonies and honeybee health in the long-term. Other factors than pesticides have been shown to be linked to overwintering success, though.

In the Netherlands, relatively high bee losses have been seen in recent years (increased mortality after winter). These losses have mainly been attributed to beekeeping practice with regard to pests and diseases, especially the *Varroa* mite, since it has been found that adequate and timely *Varroa* treatment reduces winter mortality (personal communication [bijen@wur](mailto:bijen@wur.nl) and professional beekeeper; Van der Zee & Pisa 2011). Also, reduction of forage is likely to play a role. The relationship between pesticides and bee decline has not been studied in the Netherlands so far.

A recent United Nations report (UNEP 2011) considers the status of honeybees and other pollinators worldwide. In Europe, North-America and Asia, increased bee losses have been reported. However, the symptoms seen are diverse. From Africa, reports of losses have only come from Egypt. In Australia, no increased honey bee losses have been reported (it is noted that the *Varroa* mite has not yet been introduced to this continent, except in New Zealand).

The UNEP report names many possible threats to pollinators:

- Habitat deterioration, with reduction of food sources (and habitat, for certain wild pollinators).
- Increased pathologies.
- Invasive species (the parasitic mite *Varroa destructor* is named as the most serious threat to apiculture globally).
- Pesticide use (chronic herbicide use and spray drift from broad spectrum insecticides; possible effects of chronic sublethal exposure to systemic insecticides, however this still needs to be proven in the field).
- Beekeeping activities.
- Climate change.

The conclusion of the UNEP report shows the complexity of the bee decline issue and is presented here in full:

Currently available global data and knowledge on the decline of pollinators are not sufficiently conclusive to demonstrate that there is a worldwide pollinator and related crop production crisis. Although honey bee hives have globally increased close to 45% during the last 50 years, declines have been reported in several locations, largely in Europe and Northern America. This apparent data discrepancy may be due to interpretations of local declines which may be masked by aggregated regional or global data. During the same 50-year period, agricultural production that is independent from animal pollination has doubled, while agricultural production requiring animal pollination has increased four-fold (reaching 6.1% in 2006). This appears to indicate that global agriculture has become increasingly pollinator dependant over the last 50 years. However, human activities and their environmental impacts may be detrimental to some species but beneficial to others, with sometimes subtle and counter-intuitive causal linkages. Pollination is not just a free service but one that requires investment

and stewardship to protect and sustain it. There should be a renewed focus on the study, conservation and even management of native pollinating species to complement the managed colony tradition. Economic assessments of agricultural productivity should include the costs of sustaining wild and managed pollinator populations.

Many research networks and policy programmes have been created worldwide to study and counter pollinator decline (see the UNEP report for an overview).

Based on the available information it cannot be concluded that there is a link between clothianidin and the relatively high winter mortality in honeybee colonies observed in the Netherlands in recent years. Clearly, bee decline is caused by (an interaction of) a number of factors. There is currently no evidence that clothianidin or other neonicotinoid products significantly contribute to bee decline based on public literature.

It should be noted that other (European and elsewhere) countries have not taken such steps either (with some exceptions where clear acute bee poisoning due to suboptimal sowing circumstances was observed; this has not been the case in the Netherlands).

Long-term monitoring studies investigating the effects of yearly repeated exposure on honeybee colonies to clothianidin-treated maize are still running. Final reports are expected in 2011. When these are available, Ctgb will re-assess the risk from the clothianidin products. If necessary, further monitoring may then be requested to investigate the role that clothianidin plays in bee decline, as is suggested in the 'Inclusion Directive'. At the moment, monitoring other than already ongoing is not considered necessary for clothianidin.

Cresswell (2011) has recently published a paper which questions the statistical power of honeybee field tests to show sublethal effects. This issue pertains to all pesticide risk assessments, not only to neonicotinoids, and will be considered by a European working group which has not started yet. The Netherlands will participate actively in this working group. As the impact of this paper as of yet is unclear, Ctgb will assess using the European harmonized methodologies.

Annex I. List of Endpoints Ecotoxicology

Final LoE clothianidin for inclusion in Annex I of 91/414/EEC.

For the risk assessment the final LoEP of October 2005 is used and additional data from the applicant (summarised and evaluated by Bioresearch & Promotion, Report May 2011; and by Ctgb, May and June 2011). Additions to and clarifications of the LoE are shown in italics.

Effects on honeybees (Annex IIA, point 8.3.1, Annex IIIA, point 10.4)

Acute oral toxicity

LD ₅₀ = 0.00379 µg a.s./bee LD ₅₀ = 3.9 µg TZNG/bee (<i>metabolite</i>) Metabolites TMG, MNG and TZMU: LD50 oral >100 µg /bee (<i>endpoints from DAR</i>)
--

Acute contact toxicity

LD ₅₀ = 0.04426 µg a.s./bee
--

Further laboratory tests

Chronic adult toxicity

Summarised/evaluated by Ctgb, June 2011

Kling, 2005

Acceptable: Yes

NOEC: 10 µg a.s./L sugar solution (10 d feeding).

The chronic effect of clothianidin on the honey bee, Apis mellifera L., was determined in a 10 days continuous feeding test in the laboratory. Bees (30 groups of 10 bees per test concentration and 60 groups of 10 bees in the control) were exposed to 50 % sugar solution containing four different concentrations of clothianidin by continuous and ad libitum feeding over a period of 10 days (240 hours). Mortality was observed every day. After 10 days, corrected mortality was 0.74, 14.06, 43.72 and 87.45% at 10, 20, 50 and 100 µg a.s./L, respectively. Mortality in the control was 12.35%, which was below the validity criterion of 15% set by the authors. The total intake of test item per bee accumulated over the entire test period was 0.0038 ug a.i./bee (10 ug a.i./L treatment), 0.0084 ug a.i./bee (20 ug a.i./L treatment), 0.0202 ug a.i./bee (50 ug a.i./L treatment) and 0.0485 ug a.i./bee (100 ug a.i./L treatment). No official guideline is available for this test. EPPO 2010 says that the test should be performed according to the method described in Decourtye et al. 2005. The current test is sufficiently in accordance with this method, including control mortality (Decourtye et al tested nine pesticides and found control mortality of 4-18%).

Effects on honeybee larvae

Summarised/evaluated by Bioresearch & Promotion, Report May 2011

Maus 2009

Acceptable: Yes.

NOEC: 20 µg a.s./kg diet.

Guideline: Aupinel et al (2009; draft).

Honeybee (Apis mellifera carnica) larvae were fed on an artificial diet with Clothianidin Tech. between day 4 and 6. Test rates ranged from 5 to 40 µg/kg. Mortality was determined on day +5, +6, +7, +8, +11, +13, +15 and +22.

Based on the statistical significance of the effects observed on the mortality until day +22 in three valid test runs, the study author concluded that the NOEC for this study is between 20 and 40 µg a.s./kg diet. However, differences may not be statistically significant in all runs at 40 µg a.s./kg diet, but based on graphical presentation of mean values obtained from the three valid runs there is clearly a dose related response. Also, mean mortality in the 40 µg/kg is above 30% and tests would be rejected if this had been the control mortality. Therefore it is considered that the NOEC should be set at 20 µg a.s./kg diet. The LOEC is determined to be

≥ 40 µg a.s./kg diet.

Field or semi-field tests

Several crop-dependent studies were established for maize, oilseed rape (OSR) and sunflower.

Besides these studies 2 crop-independent field studies are available in which no adverse effects were observed.

As sugar beet and fodder beet do not flower under good agricultural practice and are not favored by honeybees, no risk calculation or studies are considered necessary for this crop.

The higher tier studies mentioned above were presented in the DAR. A short summary has been added below by Ctgb. In most of these studies, residues were measured in bee-relevant matrices (honeybees, nectar, pollen). Results are given in tables copied from the DAR.

Cage tests.

Maus 2002d: Bees fed with pollen from dressed maize seeds (1 g a.s./1000 seeds) resulting in pollen containing 0.8 µg/kg. Observation period: 2 weeks. No mortality, brood and behavioural impacts related to treatment.

Schmuck & Schöning 2000a: OSR 50 g a.s./ha. Observation period: 4 days. No mortality and behavioural impacts. Residue levels in µg a.s./kg: honeybees 14; rape nectar sampled by bees: 8.6; rape nectar sampled from flowers: 1.2-7.2; rape blossoms: 4.1.

Schmuck & Schöning 2000b: OSR 50 g a.s./ha. Observation period: 4 days. No mortality and behavioural impacts. Exposure to cage in which treated and untreated plants were present. Residue levels in µg a.s./kg: honeybees <0.3; rape blossoms: 3.3.

Schmuck & Schöning 2000c: OSR 50 g a.s./ha. Observation period: 4 days. No mortality and behavioural impacts. Residue levels in µg a.s./kg: honeybees <0.3; rape nectar sampled by bees: < 10 (LOQ); rape nectar sampled from flowers: < 10 (LOQ); rape pollen sampled by bees: 1.7.

Tunnel tests

Maus & Schöning 2001b: OSR 27 g a.s./ha. Observation period: 15 days. No mortality and behavioural impacts.

Table 9.4.7-01 Summary of the analytical findings :

	Residues of TI-435 (µg/kg)	Residues of TZMU (µg/kg)	Residues of TZNG (µg/kg)
Control rape nectar A	n.d.	n.d.	n.d.
Control rape nectar B	n.d.	n.d.	n.d.
Treated rape nectar A	2.8	< LOQ	n.d.
Treated rape nectar B	3.0	n.d.	n.d.

n.d. : residues below the limit of detection (= 0.3 µg/kg for TI-435, TZMU and TZNG)

LOQ = 1µg/kg for TI-435, TZMU and TZNG

Maus & Schöning 2001c: OSR 27 g a.s./ha. Observation period: 3 weeks. No mortality and behavioural impacts.

Table 9.4.7-02 Summary of the analytical findings :

	Residues of TI-435 (µg/kg)	Residues of TZMU (µg/kg)	Residues of TZNG (µg/kg)
Control rape nectar A (capillary)	n.d.	n.d.	n.d.
Control rape nectar B (capillary)	n.d.	n.d.	n.d.
Control rape nectar C (honey comb)	n.d.	n.d.	n.d.
Treated rape nectar A (capillary)	5.4	< LOQ	n.d.
Treated rape nectar B (capillary)	1.0	n.d.	n.d.
Treated rape nectar C (honey comb)	n.d.	n.d.	n.d.
Control rape pollen A	n.d.	n.d.	n.d.
Treated rape pollen A	1.9/2.5*	n.d.	n.d.

n.d. : residues below the limit of detection (= 0.3 µg/kg for TI-435, TZMU and TZNG)

LOQ = 1µg/kg for TI-435, TZMU and TZNG

* repetition of first analysis

Maus 2002a: OSR. Residues measured at ca. 50 g a.s./ha. Effects on bees not studied.

Table 9.4.7-07 Summary of the analytical findings :

	Residues of TI-435 (mg/kg)	Residues of TZMU (mg/kg)	Residues of TZNG (mg/kg)
Control Nectar (rape blossoms)	n.d.	n.d.	n.d.
Control Nectar (honey comb)	n.d.	n.d.	n.d.
Control Pollen (honey comb)	n.d.	n.d.	n.d.
Control Leaves of Rape Blossoms	n.d.	n.d.	n.d.
Control Bees	n.d.	n.d.	n.d.
Treatment Nectar (rape blossoms)	LOQ	n.d.	n.d.
Treatment Nectar (honey comb)	LOQ	n.d.	n.d.
Treatment Pollen (honey comb)	< LOQ	n.d.	n.d.
Treatment Leaves of Rape Blossoms	< LOQ	n.d.	n.d.
Treatment Bees	< LOQ	n.d.	n.d.

n.d. : residues below the limit of detection (= 0.3 µg/kg for TI-435, TZMU and TZNG)

LOQ = 1µg/kg for TI-435, TZMU and TZNG

Maus & Schöning 2001d: Sunflower 25 g a.s./ha. Observation period: 2 weeks. No behavioural impacts. No mortality effects attributed to treatment.

Table 9.4.7-03 Summary of the analytical findings :

	Residues of TI-435 (µg/kg)	Residues of TZMU (µg/kg)	Residues of TZNG (µg/kg)
Control Sunflower Nectar A (honey comb)	n.d.	n.d.	n.d.
Treated Sunflower Nectar A (honey comb)	n.d.	n.d.	n.d.
Control Sunflower Pollen A (direct from the head)	n.d.	n.d.	n.d.
Control Sunflower Pollen B (honey comb)	n.d.	n.d.	n.d.
Treated Sunflower Pollen A (direct from the head)	2.4/3.1*	<LOQ/<LOQ*	<LOQ/n.d.*
Treated Sunflower Pollen B (honey comb)	1.2/1.3*	n.d./n.d.*	n.d./n.d.*

n.d. : residues below the limit of detection (= 0.3 µg/kg for TI-435, TZMU and TZNG)

LOQ = 1µg/kg for TI-435, TZMU and TZNG

* repetition of first analysis

Maus & Schöning 2001e: Sunflower 25 g a.s./ha. Observation period: 2 weeks. No mortality and behavioural impacts.

Table 9.4.7-04 Summary of the analytical findings :

	Residues of TI-435 (µg/kg)	Residues of TZMU (µg/kg)	Residues of TZNG (µg/kg)
Control Sunflower Nectar A (honey comb)	n.d.	n.d.	n.d.
Control Sunflower Nectar B (honey comb)	n.d.	n.d.	n.d.
Treated Sunflower Nectar A (honey comb)	n.d.	n.d.	n.d.
Treated Sunflower Nectar B (honey comb)	n.d.	n.d.	n.d.
Control Sunflower Pollen A (direct from the head)	n.d.	n.d.	n.d.
Control Sunflower Pollen B (honey comb)	n.d.	n.d.	n.d.
Treated Sunflower Pollen A (direct from the head)	2.3/2.4*	n.d./n.d.*	n.d./n.d.*
Treated Sunflower Pollen B (honey comb)	2.6/2.9*	<LOQ/n.d.*	<LOQ/n.d.*

n.d. : residues below the limit of detection (= 0.3 µg/kg for TI-435, TZMU and TZNG)

LOQ = 1µg/kg for TI-435, TZMU and TZNG

* repetition of first analysis

Maus & Schöning 2001h: Colonies fed with treated pollen at 0, 5, 10 and 20 µg a.s./kg (and untreated sunflower honey as additional food). Observation period: 2 weeks. No effects on mortality, foraging activity at the pollen and the honey feeder, brood and behaviour. NOEC: 20 µg a.s./kg pollen.

Maus & Schöning 2001i: Colonies fed with treated sunflower honey at 0, 5, 10 and 20 µg a.s./kg (and untreated pollen as additional food). Observation period: 2 weeks. No effects on mortality, foraging activity at the pollen and the honey feeder, brood and behaviour. NOEC: 20 µg a.s./kg pollen.

Field tests.

Maus & Schöning 2001a: Colonies fed with treated sugar solution. Observation period: 2 weeks. No effects on mortality, foraging activity at the syrup feeder and on behaviour. NOEC: 20 µg a.s./kg syrup.

Scott-Dupree & Spivak 2001: OSR 42 and 30 g a.s./ha. No effects on brood, foraging activity, mortality, honey production and behaviour. Observation period not given in the DAR.

Residue studies.

Maus & Schöning 2001f and 2001g: Residue studies in maize at 53.8 g a.s./ha.

Table 9.4.7-08 Summary of the analytical findings of 2 residue studies in maize (Maus Ch. & Schöning R., 2001f and 2001g)

Treatment rate : 53.8 g a.s./ha	Residues of TI-435 µg/kg	Residues of TZMU µg/kg	Residues of TZNG µg/kg
Control Maize Pollen (Test Location Farmland "Laacher Hof")	1.7/1.1/<LOQ* [§]	n.d./n.d./<LOQ*	n.d./n.d./n.d.*
Treated Maize Pollen (Test Location Farmland "Laacher Hof")	5.4/3.3*	n.d./n.d.*	n.d./n.d.*
Control Maize Pollen (Test Location Farmland "Höfchen")	3.8 [§]	n.d.	n.d.
Treated Maize Pollen (Test Location Farmland "Höfchen")	6.2	n.d.	<LOQ

n.d. : residues below the limit of detection (= 0.3 µg/kg for TI-435, TZMU and TZNG)

LOQ = 1 µg/kg for TI-435, TZMU and TZNG

* repetition of first analysis

[§] The source of this contamination could not be traced.

Maus, 2002b and 2002c: Residue studies in maize at 51.4 g a.s./ha.

Table 9.4.7-09 Summary of the analytical findings of 2 residue studies in maize (Maus Ch., 2002b and 2002c)

Treatment rate : 51.4 g a.s./ha	Residues of TI-435 µg/kg	Residues of TZMU µg/kg	Residues of TZNG µg/kg
Control Maize Pollen (Test Location Farmland "Laacher Hof")	n.d.	n.d.	n.d.
Treated Maize Pollen (Test Location Farmland "Laacher Hof")	2.4/2.9	n.d.	n.d.
Control Maize Pollen (Test Location Farmland "Höfchen")	n.d.	n.d.	n.d.
Treated Maize Pollen (Test Location Farmland "Höfchen")	2.1	n.d.	n.d.

n.d. : residues below the limit of detection (= 0.3 µg/kg for TI-435, TZMU and TZNG)

LOQ = 1 µg/kg for TI-435, TZMU and TZNG

Dust deposition sugarbeet

Summarised/evaluated by Ctgb, May 2011

Lueckmann, J. & Staedtler, T. 2009

Monitoring of dust drift deposits during and after the sowing of sugar beet pills, treated with Poncho® Beta or Poncho® Beta Plus in Germany with commercially dressed sugar beet pills (nominally 0.60 mg clothianidin & 0.08 mg beta-Cyfluthrin (+ 0.30 mg imidacloprid) per

individual sugar beet pill.

All 20 fields were sown with mechanical sowing machines. The test field sizes varied between 1.5 and 21.0 ha. Shortly before sowing, the wind direction was determined and ten Petri-dishes were placed in groups of two at a distance of 1, 3 and 5 m (in total 30 Petri-dishes) at the down-wind border of the field. To monitor a potential dust drift during a 24h-period after sowing ten new Petri-dishes were placed in pairs at the approximate middle of each field side at a distance of 1 m to the field borders. Weather conditions were presented.

The 90th percentile residue levels during the sowing operation and the 24h-sampling were all below the limit of determination (LOD 0.004 g a.s./ha). These results indicate that the dust drift produced during and after the sowing of Poncho® Beta Plus treated sugar beet pills is very limited. From these results it can be concluded that standard mechanical sowing of sugar beet pills lead to low off-crop deposition values when sown with commercial sowing equipment. This is in line with the current matrix 'Relevance of dust for pesticide treated seeds'. The conclusion in the matrix that dust formation is not relevant for sugar beet can be used for risk assessment.

Nikolakis, A., Schoening, R. 2008

Drift deposition pattern of seed treatment particles abraded from Poncho® Beta Plus treated sugar beet pills and emitted by a typical mechanical sowing machine in Germany with commercially treated sugar beet pills, treated with Poncho® Beta Plus, which contains the neonicotinoid active substances clothianidin and imidacloprid (analysed neonicotinoid seed loading: 0.589 mg clothianidin a.s./pill, 0.325 mg imidacloprid a.s./pill). The actual machine tested was a Kverneland Accord Monopill SE, a 12-row mechanical precision sugar beet planter (12 hoppers). The size of each drilling plot was about 1.0 ha with an orientation of the sampling devices 180° ± 30° to the prevailing wind direction. An average wind speed of 2 - 5 m/s and a deviation of wind direction of maximum ± 30° to the perpendicular wind direction (i.e., 180° to the sampling devices) were the target conditions during drilling. All clothianidin-containing dust and abrasion particles which deposited at 1, 3, 5, 10, 20, 30 and 50 metres distance from the drilling area during sugar beet sowing ("primary drift") were sampled in polystyrene Petri-dishes (Ø 13.7 cm, 147.41 cm²), filled with an acetonitrile-water mixture (2/8, v/v). For each sampling distance, three arrays of 10 Petri-dishes each were installed with a distance of 1 metre between the dishes and 50 m between the arrays. Passive dust-drift collectors were installed at 1 m, 2 m, 3 m, 4 m and 5 m above the soil surface. The dust collectors were made of a polypropylene fabric mesh, built up of filaments with a 0.80 × 0.18 mm cross-section. This type of collector has a slightly oval shape with a length of ≈ 85 mm and a diameter of ≈ 65 mm; at its poles, the diameter is ≈ 50 mm. The polypropylene fabric mesh collectors were pinned on each end of horizontal metal rods, which in turn were mounted at the respective height on a vertical tripod-pylon (height ≈ 6 m), giving in total 10 collectors per pylon (2 at each height). In all arrays, a pylon was installed at 5 and 30 m distance from the drilling area, respectively, resulting in 6 collectors per height per distance. Weather conditions were presented.

All 90th percentile values for ground deposition ("primary" and "secondary" drift, respectively) were at least below the limit of quantification (i.e. = LOQ = 0.014 g a.s./ha).

Considering atmospheric drift, clothianidin was measured in 75% of the passive polypropylene-mesh-collectors which were set up in different heights at 5 and 30 m distance from the sowing area. However, in contrast to ground deposition data, which are direct, area-related exposure figures [g a.s./ha], the airborne residues determined in passive samplers of an unknown collection efficiency only allow for a derivation of qualitative conclusions.

The consistent overall lack of quantifiable deposition within the off-field area suggests that airborne particles, trapped by passive polypropylene-mesh-collectors in the same area, are mainly subject to further dispersion and dilution.

These results indicate that the dust drift produced during and after the sowing of Poncho®

Beta Plus treated sugar beet pills is very limited. From these results it can be concluded that standard mechanical sowing of sugar beet pills lead to low off-crop deposition values when sown with commercial sowing equipment. This is in line with the current matrix 'Relevance of dust for pesticide treated seeds'. The conclusion in the matrix that dust formation is not relevant for sugar beet can be used for risk assessment.

Dust deposition maize

Nikolakis, A.; Casadebaig, J.; Appert, C.; Schoening, R. 2009 Summarised/evaluated by Ctgb, May 2011

Monitoring of dust drift deposits during the sowing of maize seeds, treated with Poncho® (Clothianidin FS 600) on bee health study plots in France with Poncho® (Clothianidin FS 600) treated maize seeds. The analytical verified content of clothianidin per individual maize seed was 0.50-0.51 mg a.s./maize seed.

All fields were sown with commercial vacuum-pneumatic single-kernel maize sowing machine which were modified with deflectors. Overall, four different machines with identical modification principle were used on the fields under investigation. Sowing rate was 100,000 seeds/ha. On each site of the field in 1 m distance to the sowing area, an array of 10 polystyrene Petri-dishes with an intra-row spacing of 1 m had been arranged horizontally on metal bearings at a height of approx. 1.5 to 2 cm above the soil surface or at the height of the vegetation surface, depending on the actual field boundary morphology. The actual placement of the Petri-dishes on the 4 field edges followed the actual wind direction, in order to collect as much dust as possible. Sowing parameters and environmental conditions were presented.

The maximum 90th%ile ground deposition value as determined along the four borders of each plot, respectively, was 0.092 g clothianidin a.s./ha.

Considering all plots, despite the high wind speed of plot Champagne 2 and despite a > 30 degrees wind angle, the arithmetic mean of the 90th%ile values is 0.0522 g a.s./ha. In this calculation the < LOQ value of Aquitaine plot was set to 0.014 g a.s./ha. No reference (technique) was used in the study. Only a distance of 1 m to the sowing area has been performed in the monitoring study.

In other studies (from Syngenta) evaluated by The Netherlands, the highest deposition of dust occurs at a larger distance than 1 m (see below). The downwind ground deposition is not considered a maximum conservative value for all plots because no < LOD/LOQ was measured in the Alsace and Champagne 2 plots. Therefore it is considered that a determination of a drift reduction percentage from this study cannot be performed adequately. A comparison with the other available and evaluated studies is also not possible because the distance and/or the height of the measurements is/are different. Therefore this study is not used in the risk assessment.

Nikolakis & Schoening 2008. Summary/evaluation by PRI (WUR, The Netherlands) in 2009.

Drift deposition pattern of seed treatment particles abraded from Clothianidin FS 600 dressed maize seeds and emitted by different modified and un-modified pneumatic and mechanical sowing machines.

Dust emission was studied from different maize sowing machines (vacuum pneumatic; pos/neg pressure; mechanical; with/without deflectors) and for different seed coating types. Dust drift can significantly be reduced by means of adaptations to the machine like deflectors, redirecting air towards the fertilizer bins, and redirecting exhaust air towards soil surface. Mechanical and positive air pressure maize sowing machines produce less dust drift than the standard negative pressure sowing machines. Dust drift deposit on soil surface is lower than of airborne dust drift at 1 m height at the same distance.

Other studies on dust deposition from maize sowing

The studies presented below are owned by Syngenta and were not performed with clothianidin. However, dust drift from treated seeds is not considered to be dependent on active substance. Therefore, the studies are presented below to give an overall picture of dust drift from maize seeds. The summary/evaluation was made by PRI (WUR, The Netherlands) in 2009.

In the study of Tummon, 2006 it was demonstrated that the peak of 0.55% of applied dose was found at 5 m distance (in average and in two out of 3 measurements 0.49%-0.62%).

In the study of Tummon & Jones, 2007 it was demonstrated that for the conventional sowing machine the highest dust drift deposition of dust of 0.81 % (0.80%-0.82%) occurs at 5 m distance. For the maize sowing machine using deflectors on the air exhaust pipe redirecting the air towards the seed hoppers it was demonstrated that the highest dust deposition is 0.037% (0.019%-0.24%) and occurs at 10 m distance but is still lower than the value at 50 m distance for the conventional sowing machine without air deflectors. Dust deposition decreases with increasing distance to a level of 0.004% at 50 m distance.

In the study of Solé, 2008 it was demonstrated that for the conventional sowing machine the dust drift deposition values for the two replications the highest deposition of dust of 0.99 % (0.87%-1.12%) occurs at 5 m distance.

For the maize sowing machine using dual tube deflectors on the air exhaust pipe redirecting the air towards the soil surface it was demonstrated that the highest dust drift deposition is 0.299% (0.30%-0.569%) and occurs at 10 m distance.

In conclusion, the highest drift value from maize sowing with deflectors as measured in the above studies is 0.55% of the applied dose. This value will be used in the risk assessment.

Dust toxicity

Bakker 2010. summarised/evaluated by Ctgb, May 2011 (whole summary presented below since no separate evaluation report was made)

The objective of the study was to determine the effect of clothianidin applied as dust obtained by mechanical abrasion of maize seeds treated with Clothianidin FS 600 (only particles <100 µm used) and as spray treatment with liquid formulation Clothianidin FS 600B G on the honeybee, *Apis mellifera* L., in cage tests.

30 cages of 20 m² were set up with flowering *Phacelia tanacetifolia* and one small bee colony was placed in each tunnel 6 days before the applications (5 control, 3 per treatment; 7 not used for the test except as reference for the effect of the test cages itself on colony development). Tested doses were 0.5, 2 and 4 g a.s./ha (nominal), both for the spray and dust treatment. Application was during bee flight. Control units were left untreated.

Mortality, behaviour and foraging activity were assessed daily over 7 days during the time of exposure in the tunnels. Colonies were then moved to a monitoring site for a post-exposure monitoring phase of 43 days.

Mortality was assessed with dead bee traps in front of the colony (DAA -6 till DAA +49) and on white cloth surrounding the crop, bordering the cage walls (DAA -6 till +7). Flight activity was assessed by determining the number of bees that were visiting flowers (DAA -5/3 till +7). The condition of the colonies and the development of the bee brood were assessed once before application and at DAA +7 and +28. Statistical analysis: graphically using box plots; specific hypotheses tested using repeated measures ANOVA techniques.

The results suggest that the two treatment groups (dust application and spray application) tested differed in the level of effect they caused on adult honeybee mortality. This difference

could not be related to e.g. reduced foraging in the cages treated with the spray application and therefore there is evidence that exposure to dust particles resulted in stronger effects than exposure to spray deposits with comparable amounts of active substance.

The liquid formulation was homogeneously and accurately applied to the test crop, therefore nominal rates can be used to set the spray endpoints. However, the amount of dust was variable in space, in time and with dose. This was determined in separate runs (without bees) by collecting dispersed dust on glass plates over a regular grid and assessing the dust density at the various grid points using fluorescence techniques.

Space: The large variation between individual glass plates was attributed by the author to inhomogeneous particle distribution (it is very difficult to achieve a homogeneous distribution in air of particles with settling velocities in the order of several cm/sec).

Time: There was no trend in time, implying that all dust had settled before the first collection (after 3 h) independent of dose rate.

Dose: In proportion to the equivalent of the hectare rates applied, the recovery in the three treatment regimes were 108%, 63% and 51% for 0.5, 2 and 4 g a.s./ha, respectively.

Deviations from expected deposits on individual glass plates ranged from -71% to +379% for the 0.5 g a.s./ha, from -100% to +170% for 2 g a.s./ha and from -99% to +39% to 4 g a.s./ha.

Even though the low recovery of particles at higher concentrations is not conclusively explained, it can be assumed that the average amount of particles collected on the glass plates is indicative of the average amount of particles expected on the test plants. Since all particles are expected to have settled within 3 hours, measurements of all time periods can be analyzed together to infer particle concentrations on the plants. This means that in the 0.5 g a.s./ha treatment all plants had on average a residue close to the expected dose (namely 0.54 g a.s./ha). For the 2 and 4 g a.s./ha treatments an average plant would have the equivalent of 1.26 and 2.04 g a.s./ha., respectively. However, because for each cage it is known that the exact amount corresponding to the target rate was dispersed in the cage, this finding implies that a fraction of the surface received a much higher loading. The flattening of the frequency distributions indicates that with increasing rate we do indeed expect more extreme values.

Ctgb considers that since it has not been proven that exposure in the drift study was indeed to the nominal dose of the two higher dose rates, the measured values will be used to set the dust endpoints.

Mortality was measured both in dead bee traps and on white cloths on the edges of the cages. The data from the white cloths are considered less relevant for the present study since the temperature during the study was very high. It is expected that bees would have been flying around a lot to cool off, land on the cage walls for the night and then end up dead on the white cloth in the morning, which would mask a possible treatment effect. Furthermore, dust particles will be taken into the hive and transferred from bee to bee, e.g. when dead bees are thrown out of the hive. Therefore, for the dust study the dead bee traps may show an increased effect as compared to the white cloth. For these reasons, the mortality data should be analysed for the dead bee traps and the white cloth separately and results for the dead bee traps are most important.

Dust: Mortality was statistically significantly increased in the dead bee traps at all dose rates during the exposure phase. Thus, the NOER for mortality is set at <0.5 g a.s./ha. Flight activity was not significantly reduced at any test rate: NOER flight activity: 2 g a.s./ha (actual measured).

Spray: The study author concluded that mortality was not significantly reduced at any test rate. However, from the box plots presented in the study report it is clear that there was much higher mortality in the dead bee traps at day 1 in the two highest dose rates and at day 2 at the highest dose rate. Therefore, the NOER for mortality should be set at 0.5 g a.s./ha for the spray treatment. Flight activity was significantly reduced at 4 g a.s./ha only. Thus, the NOER for flight activity is 2 g a.s./ha (nominal).

Both in the spray and the dust treatment, mortality returned to normal levels immediately after moving the hives to an uncontaminated area. No effects of the treatments on colony condition were observed. However the placement in the cages resulted in depletion of food stocks in all hives, including the control.

*Garrido 2010. Summarised/evaluated by Bioresearch & Promotion, Report May 2011
Assessment of Potential Impacts on Honey bee Colony Development and Monitoring of Aerial Dust Drift During the Sowing of Clothianidin FS 600 Treated Maize Seeds with Modified Seeding Technology Directly Adjacent to Full- Flowering Winter Oil-seed Rape in Austria.
Acceptable: Yes (but some limitations on the study conduct and data analysis were noted)*

NB This study is still running and the final report, scheduled for summer 2011, will include an assessment of the overwintering performance of the colonies.

Maize seeds treated with Clothianidin FS 600B G (612.5 g a.s./L). Nominal seed loading of 1.25 mg a.s./seed (analysed seed loading 1.285 mg a.s./seed). Furthermore treatment with fungicide Maxim® XL (active ingredients: fludioxonil and metalaxyl-M), the film coating Impranil® DLN W 50 and Talcum Gloss® powder. One treatment and one control field. During sowing (April 2010), the neighbouring oil seed rape field was in full bloom, and bees were active during applications. The sowing rate was ca 100,000 maize seeds/ha. Actual application rate 132.9 g a.s./ha. Drilling was performed with a vacuum-pneumatic maize sowing machine.

Dust was sampled with glycerol-wetted gauze netting (3 m from the field; 0.65 m height). Residues of clothianidin as determined in the gauze samples collected on the treatment field at the day of maize sowing ranged from < 1.2 mg (LOD) to 800 mg a.s./ha. Residues were distinctly higher at the downwind borders of the maize sowing area than at upwind borders. Seed-treatment dust, abraded and released during the sowing operation with modified (deflected) vacuum-pneumatic sowing equipment, resulted in a measurable off-crop exposure (in the oil seed rape field). The average vertical dust deposition corresponded to a drift rate of 420 mg a.s./ha (at the downwind side).

Mortality and colony health of eight treatment and eight control honey bee colonies were followed (mortality for three weeks, population strength and development and food stores for five months). There were no distinct differences in honey bee mortality (dead bees in bee traps) and colony strength and colony development (number of adult bees, brood development and food storage) between control and treatment. Pollen analyses showed that approximately 50% of the pollen collected were of oil seed rape, indicating that exposure of foraging bees to clothianidin dust was likely to have occurred to some extent.

The conclusion that no long term effects are induced by exposure to low levels of abraded dust from clothianidine dressed maize seeds, released during sowing, is probably correct, but it should be confirmed by statistical hypothesis testing (e.g. a pre-post repeated measures design). The addition of results regarding a firm endpoint like overwintering success will further increase the robustness of the final conclusion. Data interpretation is slightly hampered by some flaws in the experimental design (higher mean colony strength in control than in treatment before sowing; no mortality assessment pre-treatment; mortality caused by exposure to abraded dust may have been masked by the high mortality induced by the colony assessment), therefore no firm conclusions can be made regarding potential (moderate) treatment related effects on bee mortality (immediately) after sowing.

NB dust exposure was also studied in the Liepold 2010 studies (see section 'guttation and dust exposure' below).

Bee monitoring after exposure to clothianidin in dust from maize sowing

Summarised/evaluated by BioResearch & Promotion, Report May 2011

Liebig et al, 2008 and 2009.

Acceptable: Probably yes, but design and description experimental set-up is not transparent. Conclusions are probably correct (based on observations on very high number of colonies) but could not be verified (incomplete information).

The study aimed to assess duration of adverse effects induced by exposure of colonies to clothianidin residues (through several pathways):

- Affected hives from dust drift incident in Rhine Valley End of April 2008;
 - Hives located adjacent to flowering maize grown from treated seeds;
 - Effect of contaminated pollen (taken from affected hives from the dust drift incident; concentration 7 or 34 ug a.s./kg) in clean hives in clean area;
- Overwintering succes of all three exposure types was determined.

The conclusion of the study authors are presented below. Due to flaws in experimental design or incomplete or unclear information, some of the conclusions drawn in these studies are not fully supported:

1) Exposure to dust during sowing of maize seeds has adverse effects on bee colony development, but colonies can recover, and successfully overwinter.

Objections:

- The information provided in the study does not show that colonies were indeed affected.
- Pollen combs with contaminated pollen were removed from these test hives, and the conditions of these hives were therefore not representative for conditions of bee colonies residing in areas contaminated with maize seed dust.
- Healthy colonies did not clearly suffer from consumption of the contaminated pollen (the exposure to contaminated pollen was not worst case; a dilution effect may have occurred. However, the tested situation is representative for areas in which not only maize fields are present). The effect of the contaminated pollen on affected colonies was not tested (at least not from worst-case exposure; part of the contaminated was pollen was taken from the colonies and it is not clear from the report how much was left).
- Information from the 2009 report indicates that winter mortality at a site in Upper Swabia "damaged by maize sowing" was 30% (3 out of 10). This means that the conclusion of the study author that recovery and succesfull overwintering occurred after dust damage, is not correct at least for this locality.

2) Bee colonies foraging on the edge of maize fields grown from treated seeds were not adversely affected in terms of colony health and overwintering success. In fact treatment colonies performed much better than the controls.

Objections:

- Comparisons to the control are not useful, because test and control colonies were not of the same origin, and resided at locations far away from the test site (ca 50-70 km) under incomparable (climatic) conditions.

3) Because of an overall winter mortality in Baden-Wurtemberg of 4% it was concluded that damage to colonies due to sowing of flawed maize was only temporary.

Objections:

- It is not clear from the study description which fraction of the monitored colonies was in contaminated areas.

Long-term studies after exposure to flowering maize

(Interim reports; the studies are still running and the final reports are scheduled for summer/autumn 2011).

Hecht-Rost 2009. Assessment of Side Effects of Clothianidin FS 600B G Treated Maize Seed on the Honeybee (*Apis mellifera* L.) in a Long-Term Field Study in Alsace (France).

Acceptable: Yes (but some limitations on the data analysis and interpretation were noted)

The study was conducted on commercial agricultural land near La Petite Pierre, Alsace, France. There was one treatment field (1.4 ha) and one control field (1.8 ha), located at approximately 4 km distance. Fields were sown in May 2008 with maize. The control treatment consisted of a field sown with untreated maize seeds.

Maize seeds for the treatment field were dressed with Clothianidin FS 600B G at a nominal seed loading of 0.5 mg a.s./seed. Seeds were drilled in spring 2008 at a rate of 30 kg seeds/ha (application rate not reported but estimated at 60 g a.s./ha).

In each treatment group six colonies were placed for 11 days (summer 2008) at the border of the test fields with flowering maize. Thereafter colonies were kept for monitoring in a remote site without extensive agricultural crops attractive to the bees.

There were no distinct treatment related differences in behaviour (flight and foraging activity) and mortality (dead bees in bee traps and linen sheets) (measured during the 11-d exposure period); and colony strength and colony development (number of adult bees, brood development and food storage; measured three weekly before and once after overwintering) in the first year of observation after exposure to flowering maize grown from clothianidin treated seeds. Low residue levels were found in pollen from bees (3 µg/kg) and from plants (5 µg/kg) in the treatment group but not in the control group. Pollen were collected on the 5th and 6th exposure day from pollen traps attached to the hive entrance of each hive. The mean fraction of maize pollen was 1-3% on the 5th day and 7-9% on the 6th day in both treatment groups.

Results indicate that there are no long term effects induced by exposure to flowering maize grown from clothianidin dressed maize seeds. However, this conclusion was not supported by statistical hypothesis testing. The low fraction of maize pollen collected by bees and the low contents of clothianidin in the analysed pollen samples signifies low exposure in this study, which makes the study not realistic worse case. Colony strength after/before overwintering was approximately 0.5 in both groups, which is rather low.

Unclear effects on flight activity (higher in the treatment group than in the control) and bee mortality (higher in the control) are not adequately explained, but are probably of minor importance since no long term effects were apparent.

Hecht-Rost 2008. Assessment of Side Effects of Clothianidin FS 600B G Treated Maize Seed on the Honeybee (*Apis mellifera* L.) in a Long-Term Field Study in Champagne (France).

Acceptable: Yes (but some limitations on the data analysis and interpretation were noted)

The study was conducted on commercial agricultural land near Chalons en Champagne, Champagne, France. There was one treatment field (2.0 ha) and one control field (1.9 ha), located at approximately 2 km distance. Fields were sown in spring 2008 with maize. The control treatment consisted of a field sown with untreated maize seeds.

Maize seeds for the treatment field were dressed with Clothianidin FS 600B G at a nominal seed loading of 0.5 mg a.s./seed. Seeds were drilled in spring 2008 at a rate of 30 kg seeds/ha (application rate not reported but estimated at 60 g a.s./ha).

In each treatment group six colonies were placed for 10 days (summer 2008) at the border of the test fields with flowering maize. Thereafter colonies were kept for monitoring in a remote site without extensive agricultural crops attractive to the bees.

There were no distinct treatment related differences in behaviour (flight and foraging activity) and mortality (dead bees in bee traps and linen sheets) (measured during the 10-d exposure period); and colony strength and colony development (number of adult bees, brood

development and food storage; measured three weekly before and once after overwintering) in the first year of observation after exposure to flowering maize grown from clothianidin treated seeds. Low residue levels were found in pollen from plants (1 µg/kg) in the treatment group but not in the control group. Pollen were collected on the 2nd and 3rd exposure day from pollen traps attached to the hive entrance of each hive. The mean fraction of maize pollen was 0-1% in the control groups and 1-4% (excluding one finding of 21% on one day in a very small sample) in the treatment group. The amount of pollen collected from foraging bees was too small for chemical analysis.

Results indicate that there are no long term effects induced by exposure to flowering maize grown from clothianidin dressed maize seeds. However, this conclusion was not supported by statistical hypothesis testing. The low fraction of maize pollen collected by bees and the low contents of clothianidin in the analysed pollen samples signifies low exposure in this study, which makes the study not a realistic worst case.

Unclear effects on flight activity (higher in the treatment group than in the control) and bee mortality (increased mortality in the treatment group on exposure days 7 and 8 were attributed to robbery of one colony, but this seems unlikely because this colony was the largest colony at that time of the study) are not adequately explained, but are probably of minor importance since no long term effects were apparent.

Hecht-Rost 2008. Assessment of Side Effects of Clothianidin FS 600B G Treated Maize Seed on the Honeybee (*Apis mellifera* L.) in a Long-Term Field Study in Languedoc-Roussillon (France).

Acceptable: **No**. Conclusions are doubted. Re-evaluation of the data is considered necessary

The study was conducted on commercial agricultural land near Meynes, Languedoc-Roussillon, France. There was one treatment field (2.1 ha) and one control field (3.2 ha), located at approximately 2.5 km distance. Fields were sown in spring 2008 with maize. The control treatment consisted of a field sown with untreated maize seeds.

Maize seeds for the treatment field were dressed with Clothianidin FS 600B G at a nominal seed loading of 0.5 mg a.s./seed. Seeds were drilled in spring 2008 at a rate of 30 kg seeds/ha (application rate not reported but estimated at 60 g a.s./ha).

In each treatment group six colonies were placed for 10 days (summer 2008) at the border of the test fields with flowering maize. Thereafter colonies were kept for monitoring in a remote site without extensive agricultural crops attractive to the bees.

There were no distinct treatment related differences in behaviour (flight and foraging activity) and mortality (dead bees in bee traps and linen sheets) (measured during the 10-d exposure period); and colony strength and colony development (number of adult bees, brood development and food storage; measured three weekly before and once after overwintering) in the first year of observation after exposure to flowering maize grown from clothianidin treated seeds. Low residue levels were found in pollen from bees and plants (5 µg/kg) in the treatment group but not in the control group. Pollen were collected on the 2nd and 3rd exposure day from pollen traps attached to the hive entrance of each hive. The mean fraction of maize pollen was 7-36% in the control groups and 16-99% in the treatment group.

The proportion of maize pollen collected by the bees was much higher in this study than in the two studies conducted in the North (Alsace and Champagne, see above), hence exposure in this study is likely to have been higher compared to both North studies.

According to the study author there are no long term effects induced by exposure to flowering maize grown from clothianidin dressed maize seeds. However, this conclusion is not accepted. Study results indicate that there may be adverse treatment related effects on colony development (a consistent trend was observed that mean colony strength gradually decreased over time in the treatment group, whereas strength in the control group remained constant

after a slight increase). Despite this graphically observed difference between the two test groups, it is concluded by the study authors that “a treatment effect is not to be assumed behind this figure”. However, re-analyses of the data is considered necessary before proper conclusions can be drawn. Reconsideration of the acceptability of the disease level and possible exclusion of individual colonies from analyses may lead to an overall rejection of the study if insufficient data remain for evaluation.

Disease analysis of the three Hecht-Rost studies (prepared by study evaluator)

Nosema and Varroa were found in all three studies. Nosema had no expected influence on overwintering success in both treatment and control groups. The influence of Varroa on colony strength could not be checked. Furthermore, chalkbrood and deformed wing virus both occurred in the Languedoc-Roussillon study. It cannot be excluded that the combination of diseases observed in the Languedoc-Roussillon study may have influenced certain parameter values. As far as could be deduced from the data provided, the occurrence of the diseases was probably biased towards one of the groups and may have further increased variability, thereby masking potential treatment related effects.

see also Liepold 2010 below

Guttation and dust exposure - maize

Liepold 2010 - Alsace

Acceptable: Guttation yes. For other parameters (mortality, colony health) re-evaluation of the data is considered necessary before conclusions can be drawn.

The study was conducted on commercial agricultural land in Alsace, France. There was one treatment field (1.8 ha) and one control field (2.8 ha), located at approximately 4 km distance. Maize seeds for the treatment field were dressed with a nominal seed loading of 0.5 mg a.s./seed. Seeds were drilled at a rate of 100,000 seeds/ha (dose rate 50 g a.s./ha). The control treatment consisted of a field sown with untreated maize seeds. A Monosem NG 3 plus (6 rows) with Syngenta reflector kit was used for drilling (precision planter using vacuum metering system).

Six colonies per field were placed at the border of the test fields 19 days before seed drilling and kept there for 62 days, after which they were transferred to a monitoring site. In total colonies were monitored for 113 days.

For 53 days after emergence of the seedlings, the occurrence of guttation was observed systematically by inspecting the field daily several times from ca 6-7 to ca 12-13 hr in 5 different observation zones (1 off- and 4 in-field). In each zone, the number of bees resting on plants or on the ground, and the number of bees actually making contact with guttation fluid was recorded. In addition, the proportion of guttating plants in all rows of the observation zones was estimated using a classification scheme. Furthermore the occurrence of dew was recorded.

Guttation was observed to take place in the morning on the majority of observation days, and timing during the day partly overlapped with the period of high flight activity of the bees. Bees were never observed to collect guttation fluid, and seldom were they seen in contact with guttating plants.

The study sufficiently demonstrated that exposure to and consumption of guttation fluid by foraging bees is unlikely to happen, or only at a very low rate. However, results for bee mortality, flight activity and colony assessments were not accepted. Results concerning bee mortality and colony health were influenced by flaws in the experimental set-up, inferior health of some of the test colonies (3 out of 12 colonies died during the experiment), and unforeseen causes (robbing, which also indicates weakness of the colonies). Furthermore, data may be influenced by systematic errors in assessment procedures (as in the Languedoc-Roussillon

and Aquitaine studies below).

It is not likely that exposure to dust during and shortly after drilling has had considerable effects on bee mortality or colony development during the exposure phase, but re-analyses of the data is considered necessary before proper conclusions can be drawn. Reconsideration of the acceptability of the health status and possible exclusion of individual colonies from analyses may lead to an overall rejection of the study if insufficient data remain for evaluation.

Liepold 2010 - Champagne

Acceptable: Guttation yes. No short term effects on bee endpoints during exposure phase. Re-evaluation of the data is considered necessary before conclusions can be drawn about long term effects.

The study was conducted on commercial agricultural land in Champagne, France. There was one treatment field (1.7 ha) and one control field (1.9 ha), located at approximately 2 km distance.

Maize seeds for the treatment field were dressed with a nominal seed loading of 0.5 mg a.s./seed. Seeds were drilled at a rate of 100,000 seeds/ha (dose rate 50 g a.s./ha). The control treatment consisted of a field sown with untreated maize seeds. A Monosem PNU (4 rows) with Monosem reflector kit was used for drilling (precision planter using vacuum metering system).

Six colonies per field were placed at the border of the test fields 11 days before seed drilling and kept there for 35 days, after which they were transferred to a monitoring site. In total colonies were monitored for 68 days.

For 24 days after emergence of the seedlings, the occurrence of guttation was observed systematically by inspecting the field daily several times from ca 6-7 to ca 12-13 hr in 5 different observation zones (1 off- and 4 in-field). In each zone, the number of bees resting on plants or on the ground, and the number of bees actually making contact with guttation fluid was recorded. In addition, the proportion of guttating plants in all rows of the observation zones was estimated using a classification scheme. Furthermore the occurrence of dew was recorded.

Guttation was observed to take place in the morning on the majority of observation days, and timing during the day partly overlapped with the period of high flight activity of the bees. Bees were never observed to collect guttation fluid, and seldom were they seen in contact with guttating plants.

The study sufficiently demonstrated that exposure to and consumption of guttation fluid by foraging bees is unlikely to happen, or only at a very low rate. It is also demonstrated that maize seed drilling and guttation of seedlings had no short term effects on bee colony development during the exposure period (24 days). However, results for bee mortality and colony assessments in the longer-term (after 68 days) were not accepted. Results concerning bee mortality and colony health were influenced by some flaws in the experimental set-up and unforeseen natural causes (swarming, probably in three colonies, one of which died). The dramatic decrease in colony strength in all colonies after relocation should be explained. Observations from colonies T4, T6 and C4 after relocation should be removed from the dataset for a better presentation of data obtained during the monitoring period.

Liepold 2010 – Languedoc-Roussillon

Acceptable: Guttation yes. Probably no effects on bee endpoints, but data interpretation hampered by systematic errors in assessments

The study was conducted on commercial agricultural land in Languedoc-Roussillon, France. There was one treatment field (2.1 ha) and one control field (2.8 ha), located at approximately 3.3 km distance.

Maize seeds for the treatment field were dressed with a nominal seed loading of 0.5 mg

a.s./seed. Seeds were drilled at a rate of 100,000 seeds/ha (dose rate 50 g a.s./ha). The control treatment consisted of a field sown with untreated maize seeds. A Nodet Pneumasem II (4 rows) with Bayer reflector kit was used for drilling (precision planter using vacuum metering system).

Six colonies per field were placed at the border of the test fields 4 days before seed drilling and kept there for 40 days, after which they were transferred to a monitoring site. In total colonies were monitored for 77 days.

For 24 days after emergence of the seedlings, the occurrence of guttation was observed systematically by inspecting the field daily several times from ca 6-7 to ca 10-11 hr in 5 different observation zones (1 off- and 4 in-field). In each zone, the number of bees resting on plants or on the ground, and the number of bees actually making contact with guttation fluid was recorded. In addition, the proportion of guttating plants in all rows of the observation zones was estimated using a classification scheme. Furthermore the occurrence of dew was recorded.

Guttation was observed to take place in the morning on the majority of observation days, and timing during the day partly overlapped with the period of high flight activity of the bees. Bees were never observed to collect guttation fluid, and seldom were they seen in contact with guttating plants.

The study sufficiently demonstrated that exposure to and consumption of guttation fluid by foraging bees is unlikely to happen, or only at a very low rate. The study outline (aim, experimental set-up and results) is well described, but results concerning bee mortality and colony health were influenced by flaws in the study design and systematic errors in assessment procedures.

It is probably correct that observed differences are not related to treatment, but with the observed magnitude of effects induced by systematic error, it is believed that the test-system is not sufficiently robust to detect potential treatment related effects of moderate size.

Liepold 2010 – Aquitaine

Acceptable: Guttation yes. Probably no effects on bee endpoints, but data interpretation hampered by systematic errors in assessments and sub-optimal health conditions of test colonies.

The study was conducted on commercial agricultural land in Languedoc-Roussillon, France. There was one treatment field (2.2 ha) and one control field (2.3 ha), located at 6.7 km distance.

Maize seeds for the treatment field were dressed with a nominal seed loading of 0.5 mg a.s./seed. Seeds were drilled at a rate of 100,000 seeds/ha (dose rate 50 g a.s./ha). The control treatment consisted of a field sown with untreated maize seeds. A Gaspardo MT (4 rows) with Gaspardo reflector kit was used for drilling (precision planter using vacuum metering system).

Six colonies per field were placed at the border of the test fields 6 days before seed drilling and kept there for 65 days, after which they were transferred to a monitoring site. In total colonies were monitored for 119 days.

For 24 days after emergence of the seedlings, the occurrence of guttation was observed systematically by inspecting the field daily several times from ca 6-7 to ca 12 hr in 5 different observation zones (1 off- and 4 in-field). In each zone, the number of bees resting on plants or on the ground, and the number of bees actually making contact with guttation fluid was recorded. In addition, the proportion of guttating plants in all rows of the observation zones was estimated using a classification scheme. Furthermore the occurrence of dew was recorded.

Guttation was observed to take place in the morning on the majority of observation days, and timing during the day partly overlapped with the period of high flight activity of the bees. Bees were never observed to collect guttation fluid, and seldom were they seen in contact with guttating plants.

The study sufficiently demonstrated that exposure to and consumption of guttation fluid by foraging bees is unlikely to happen, or only at a very low rate. It is not likely that exposure to dust during and shortly after drilling has had considerable effects on bee mortality or colony development during the exposure phase, but no firm conclusions can be drawn from the data obtained in this study. Results are influenced by systematic error during assessment procedures and suboptimal health conditions of the test organisms. It is believed that the experimental set-up used in this study is not sufficiently robust to detect potential moderate treatment related effects.

NB Residues in guttation droplets, bees, pollen and nectar were not analysed in any of the Liepold studies.

Lueckmann et al, 2010.

Acceptable: Guttation yes. Probably no effects on bee endpoints, but data interpretation hampered by sub-optimal health conditions of test colonies and the lack of a control reference.

The study was conducted on commercial agricultural land at several locations in two Austrian regions, north of the Alps (Baumgartenberg) and south of the Alps (Jennersdorf). In each of these regions 15 maize fields were monitored, each with two bee hives (hence 60 colonies in total). Test fields were usually larger than 2 ha (five fields were 0.4 to 1.5 ha). Maize seeds were dressed with Poncho® (0.5 mg clothianidin./seed), except three fields in the Jennersdorf region with Poncho Pro® (1.25 mg clothianidin /seed). Drilling rate was approximately 80-90 thousand seeds/ha in all fields. Different drilling machines were used for sowing, but they were all precision planters using vacuum metering system (the use of a deflector kit was not reported).

Drinking places (e.g. ditches, streams, ponds) for bees were usually more than 300 m away from the experimental fields. To 5 out of 15 fields in each region an artificial water source was offered directly adjacent to the hives (a plastic trough filled with gravel to avoid drowning). Water was slightly salted to increase attractiveness. Water was replenished at least weekly.

There were no control sites.

In each field two colonies were placed at the border of the test fields 2 to 5 days after seed drilling. Due to unexpected low colony strength several colonies were replaced 10-12 days after drilling. Monitoring started 0-4 days after emergence of seedlings. Field observations lasted approximately 3 (Jennersdorf) to 6 (Baumgartenberg) weeks. Thereafter, colonies were relocated to a monitoring site for further brood development assessments. In total, colonies were observed for about 2 months.

The occurrence of guttation was observed systematically by inspecting the field daily several times starting from sunrise till sunset in 5 different observation zones, for 3-6 weeks.

Residues in guttation droplets were measured. Initial concentrations of clothianidin (<one week after emergence) were in the range of 100-200 mg/L. Samples suspected to be diluted with dew, rain or leaf axil fluid showed much lower concentrations. Residue levels decreased exponentially to 1 mg/L and 0.01 mg/L three weeks and five weeks after emergence, respectively. No clear differences in concentrations were found for samples collected in fields treated with Poncho® or Poncho Pro®.

Daily mortality (dead bees in the bee traps) was generally below 20, except for some occurrences of peak mortality.

For the Baumgartenberg region the number of days with increased mortality was statistically significantly lower in colonies with a water supply compared to colonies without this water source in close vicinity of the hive. For colonies in Jennersdorf this relation was not found.

Residues were found on dead bees, but levels were not correlated with mortality rates or to the absence/presence of water supplies.

The study demonstrated that honey bees do occasionally use guttation fluid as drinking supply, and guttation does contain considerable amounts of clothianidin, diminishing over time,

but guttation is not a favoured water source, and mortality of adult bees measured at the hives was generally low, confirming that potential exposure to and/or uptake of contaminated guttation fluid did not lead to noticeable increases of adult bee mortality measured at the hive. The study also indicates that no measurable long-term influences on colony health occurred. But the overall sub-optimal and variable colony conditions, and the lack of a proper control reference or a more firm endpoint like overwintering success makes this expert statement not very robust.

Bees exposed to guttation fluid (starting a few days after drilling) are also likely to be exposed to dust released during drilling. This potential exposure mode was (partly or entirely) excluded from the experimental set-up used in this study.

Guttation in sugarbeet

Summarised/evaluated by BioResearch & Promotion, Report May 2011

Kepler, 2009

Acceptable: yes.

The occurrence of guttation was recorded in twelve commercial sugar beet fields and its adjacent crops or habitats, in a typical German sugar beet growing area (North-Rhine). Observations were made in early morning (between 6.10 and 8.30 h) in April/May 2009, at BBCH 10-12 (up to 2 leaves unfolded) to BBCH 14-19 (4-9 leaves unfolded). According to the author, out of 98 visits guttation was observed once (1%) in sugar beet, whereas on 83% of the visits guttation was observed in the adjacent crops/habitats. The author excluded the occurrences that droplets were observed on sugar beet leaves on days with intensive dewfall (16%), since droplets and guttation could not be distinguished. However, this exclusion was not done in adjacent crops/habitat.

Despite this weakness in analysis, the risk via guttation is considered to be low. Due to dangers (e.g. presence of predators) bees are not keen on foraging on plants unless there is a considerable reward (pollen, nectar). Therefore, drinking droplets from plants is not likely to occur (personal communication of a professional beekeeper). The risk for bees to be exposed to contaminated guttation fluid is therefore low, regardless of the frequency of guttation to occur (1% or 16%).

Residues

Summarised/evaluated by BioResearch & Promotion, Report May 2011

Note that residues were also measured in studies which were included in the DAR; see heading Field or semi-field tests above.

Staedtler 2008

Acceptable: Yes, but it is unclear whether results obtained from this study are representative under all sowing circumstances.

The study aimed to assess the amount of clothianidin residues and its metabolites (TZMU=Thiazolymethylurea and TZNG=Thiazolynitroguanidine) in maize pollen taken from plants grown in the Upper Rhine Valley from commercial seeds dressed with Poncho Pro® (a.s. clothianidin, nominally 1.25 mg a.s./seed). Additional assessments were made on pollen collected by bees from pollen traps, from bee bread and from dead bees discarded from the hive.

LOQ for clothianidin, TZMU and TZNG (the lowest validated fortification level): 1 µg/kg for all material types. LOD: 0.3 µg/kg (all material types)

From all pollen samples analyzed (pollen collected from plants, 252 samples) 31% contained clothianidin at a rate between 1 and 3 µg/kg and 62% between 3 and 10 µg/kg. One sample with 10.4 µg/kg was found, other samples (6%) were below the LOQ.

The occurrence of clothianidin residues in pollen collected by bees was much lower. From 119

samples analyzed 19% contained clothianidin at a rate between 1 and 3 µg/kg and 3% between 3 and 10 µg/kg. One measurement of 11.4 µg/kg was made. Clothianidin concentrations in bee bread above 3 µg/kg were measured only twice (n=37).

Clothianidin was found on dead bees in 8 out of 38 samples (19%), but concentrations were above 1 µg (below 3 µg) in two samples only (5%).

Concentrations of the derivatives in all sample types were almost always below the LOQ (1 µg/kg).

Between the three locations where bee-derived matrices were collected some differences were observed, but these were usually below a factor 2.

Mean and maximum values of clothianidin and its derivatives measured in this study are presented in the table below (in µg/kg).

a.s. content in µg/kg	clothianidin		TZNG		TZMU	
	mean	maximum	mean	maximum	mean	maximum
Pollen from field	3.4	10.4	0.6	1.0	0.4	<LOQ
Pollen from bees	1.1	11.4	0.4	1.8	0.3	<LOQ
Bee bread	1.0	3.3	0.4	1.3	<LOD	<LOD
Dead bees	0.5	1.2	0.4	1.2	<LOD	<LOD

Because the sowing part of this study was not described, not conducted under GLP and not performed by the testing facility, it is difficult to assess whether the sowing circumstances are relevant for all circumstances (e.g. seed dressing method, maize variety, sowing operating machinery, sowing period, weather conditions).

Annex II. Public literature

A public literature survey on the effects of neonicotinoids and fipronil on bee mortality and decline is in development under the authority of the Ministry of Economy, Agriculture and Innovation (EL&I). The preliminary results of this survey have been used for this risk assessment. Literature consulted is shown below.

Literatuur

- Alaux C, Brunet J-L, Dussaubat C, Mondet F, Tchamitchan S, Cousin M, Brillard J, Baldy A, Belzunces LP & LeConte Y, 2010. Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environm. Microbiology* 12(3),774-782.
- Alaux C, F Ducloz, D Crauser & Y Le Conte 2010. Diet effects on honeybee immunocompetence. *Biology Letters* online doi: 10.1098/rsbl.2009.0986
- Aliouane Y, Adessalam K, El Hassani AK, Gary V, Armengaud C, Lambin M, Gauthier M. 2009. Subchronic exposure of honeybees to sublethal doses of pesticides: effect on behavior. *Environ Toxicol Chem* 28: 113-122.
- Bacandritsos N, Granato A, Budge G, Papanastasiou I, Roinioti E, Caldon M, Falcaro C, Gallina A, Mutinelli F. 2010. Sudden deaths and colony population decline in Greek honey bee colonies. *Journal of Invertebrate Pathology* 105:335-340.
- Bailey J, Scott-Dupree C, Harris R, Tolman J, Harris B. 2005. Contact and oral toxicity to honey bees (*Apis mellifera*) of agents registered for use for sweet corn insect control in Ontario, Canada. *Apidologie* 36: 623-633.
- Bernadou A, Démares F, Couret-Fauvel T, Sandoz JC, Gauthier M. 2009. Effect of fipronil on side-specific antennal tactile learning in the honeybee. *J Insect Physiol*: 1099-1106.
- Bernal J, Garrido-Bailon E, del Nozal MJ, Gonzalez-Porto AV, Martin-Hernandez R, Diego JC, Jimenez JJ, Bernal JL, Higes M. 2010. Overview of pesticide residues in stored pollen and

- their potential effect on bee colony (*Apis mellifera*) losses in Spain. *Journal of Economic Entomology* 103:1964-1971.
- Bernal J, Martin-hernandez R, Diego JC, Nozal MJ, Gozalez-Porto AV, Bernal JL & Higes M, 2011. An exposure study to assess the potential impact of fipronil in treated sunflower seeds on honey bee colony losses in Spain. *Pest Manag Sci* on line, DOI10.1002/ps.2188
- Bonmatin JM, Moineau I, Charvet R, Fleche C, Colin ME, Bengsch ER. 2003. A LC/APCI-MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens. *Analytical Chemistry* 75:2027-2033.
- Bonmatin JM, PA Marchand, R Charvet, I Moineau, ER Bengsch & ME Colin 2005. Quantification of imidacloprid uptake in maize crops. *J. Agric Food Chem* 53, 5336-41
- Bortolotti, L, Montanari R, Marcelino J, Medrzycki P, Maini S & Porrini C 2003. Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. *Bulletin of Insectology* 56, 63-67
- Brunet JL, Badiou A, Belzunces LP. 2005. In vivo metabolic fate of [C-14]-acetamiprid in six biological compartments of the honeybee, *Apis mellifera* L. *Pest Management Science* 61:742-748.
- Charvet R, Katouzian-Safadi M, Colin ME, Marchand PA, Bonmatin JM. 2004. Systemic insecticides: New risk for pollinator insects. *Annales Pharmaceutiques Francaises* 62:29-35.
- Chaton PF, Ravanel P, Meyran JC, Tissut M. 2001. The toxicological effects and bioaccumulation of fipronil in larvae of the mosquito *Aedes aegypti* in aqueous medium. *Pesticide Biochemistry and Physiology* 69:183-188.
- Chauzat MP, Carpentier P, Martel AC, Bougeard S, Cougoule N, Porta P, Lachaize J, Madec F, Aubert M, Faucon JP. 2009. Influence of pesticide residues on honey bee (Hymenoptera: Apidae) colony health in France. *Environmental Entomology* 38:514-523.
- Chauzat MP, Faucon JP, Martel AC, Lachaize J, Cougoule N, Aubert M. 2006. A survey of pesticide residues in pollen loads collected by honey bees in France. *Journal of Economic Entomology* 99:253-262.
- Chauzat MP, Martel AC, Cougoule N, Porta P, Lachaize J, Zeggane S, Aubert M, Carpentier P, Faucon JP. 2011. An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera Apidae) to monitor pesticide presences in continental France. *Environmental Toxicology and Chemistry* 30:103-111.
- Chauzat, M. P., J. P. Faucon, A. C. Martel, J. Lachaize, N. Cougoule, and M. Aubert. 2006. A survey on pesticide residues in pollen loads collected by honey-bees (*Apis mellifera*) in France. *J. Econ. Entomol.* 99: 253-262.
- Chauzat, MP, Carpentier P, Martel AM, Bougeard S, Cougoule N, Porta P, LaChaize J, Madec F, Aubert M & Faucon JP 2009. Influence of Pesticide Residues on Honey Bee (Hymenoptera: Apidae) Colony Health in France. *Environ. Entomol.* 38(3): 514-523
- Choudhary A, Sharma DC. 2008. Dynamics of pesticide residues in nectar and pollen of mustard (*Brassica juncea* (L.) Czern.) grown in Himachal Pradesh (India). *Environmental Monitoring and Assessment* 144:143-150.
- Comité Scientifique et Technique de l'Etude Multifactorielle des Troubles des abeilles (CST), 2003. Imidaclopride utilisé en enrobage de semences (Gaucho®) et troubles des abeilles. Rapport final. 106 pp.
- Cresswell JE (1999) The influence of nectar and pollen availability on pollen transfer by individual flowers of oil-seed rape (*Brassica napus*) when pollinated by bumblebees (*Bombus lapidarius*). *J Ecol* 87:670-677
- Cresswell JE. 2011. A meta-analysis of experiments testing the effects of neonicotinoid insecticide (imidacloprid) on honey bees. *Ecotoxicology* 20: 149-157.
- Cutler GC & Scott-Dupree CD, 2007. Exposure to Clothianidin seed treated canola has no long-term impact on honey bees. *J. Econ. Entomol* 100, 765-772
- Cutler GC, Scott-Dupree CD. 2007. Exposure to clothianidin seed-treated canola has no long-term impact on honey bees. *Journal of Economic Entomology* 100:765-772.
- De la Rúa P., R. Jaffé, R. Dall'Olio, I. Muñoz & J. Serrano 2009. Biodiversity, conservation and current threats to European honeybees. *Review. Apidologie* 40, 263-284

- Decourtye A & Devillers J 2010. Ecotoxicity of neonicotinoid insecticides to bees. *In*: ST Thany (ed.) "Insect nicotinic acetylcholine receptors" Landes Bioscience and Springer Science + Business media. pp. 85-95.
- Decourtye A, Armengaud C, Renou M, Devillers J, Cluzeau S, Gauthier M, Pham-Delègue M-H. 2004b. Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). *Pestic Biochem Physiol* 78: 83-92.
- Decourtye A, Devillers J, Aupinel P, Brun F, Bagnis C, Fourrier J, Gauthier M. 2011. Honeybee tracking with microchips: a new methodology to measure the effects of pesticides. *Ecotoxicology* 20: 429-437.
- Decourtye A, Devillers J, Cluzeau S et al. 2004a. Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. *Ecotoxicol Environ Saf* 57: 410-419.
- Decourtye A, Devillers J, Genecque E, [Le Menach K](#), [Budzinski H](#), [Cluzeau S](#), [Pham-Delegue MH](#). 2005. Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. *Arch Environ Contam Toxicol* 48: 242-250.
- Decourtye A, Lacassie E, Pham-Delegue MH. 2003. Learning performances of honeybees (*Apis mellifera* L.) are differentially affected by imidacloprid according to the season. *Pest Manag Sci* 59: 269-278.
- Decourtye A, Le Metayer M, Pottiau H, Tisseur M, Odoux JF, Pham-Delegue MH. 2001. Impairment of olfactory learning performances in the honey bee after long term ingestion of imidacloprid. *Hazard of Pesticides to Bees*, 113-117.
- Decourtye A, Mader E, Desneux N, 2010 Landscape enhancement of floral resources for honey bees in agro-ecosystems. *Apidologie* 41, 264–277
- Durham EW, Siegfried BD, Scharf ME. 2002. In vivo and in vitro metabolism of fipronil by larvae of the European corn borer *Ostrinia nubilalis*. *Pest Management Science* 58:799-804.
- El Hassani AK, Dacher M, Garry V et al. 2008. Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of the honeybee (*Apis mellifera*). *Arch Environ Contam Toxicol* 54: 653-661.
- [El Hassani AK](#), [Dacher M](#), [Gauthier M](#), [Armengaud C](#). 2005. Effects of sublethal doses of fipronil on the behavior of the honeybee (*Apis mellifera*). *Pharmacol Biochem Behav* 82: 30-39.
- El Hassani AK, Dupuis JP, Gauthier M, Armengaud C. 2009. Glutamatergic and GABAergic effects of fipronil on olfactory learning and memory in the honeybee. *Invert Neurosci* 9: 91-100.
- Elbert C, Erdelen C, Kuehnhold J, Nauen R, Schmidt HW, Hattori Y. 2000. Thiocloprid: a novel neonicotinoids insecticide for foliar application. Brighton Crop Protection Conference, Brighton, UK. *Pest and Diseases* 2(a): 21-26.
- Fang Q, Huang CH, Ye GY, Yao HW, Cheng JA, Akhtar ZR. 2008. Differential fipronil susceptibility and metabolism in two rice stem borers from China. *Journal of Economic Entomology* 101:1415-1420.
- Faucon J-P, Aurières C, Drajnudel P, Mathieu L, Ribière M, Martel A-C, Zeggane S, Chauzat M-P, Aubert MFA. 2005. Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies. *Pest Manag Sci* 61: 111-125.
- Faucon, J. P., C. Aurières, P. Drajnudel, L. Mathieu, M. Ribière, A. C. Martel, S. Zeggane, M. P. Chauzat, and M. Aubert. 2005. Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies. *Pest Manag. Sci.* 61: 111-125
- Garcia-Chao M, Jesus Agruna M, Flores Calvete G, Sakkas V, Llompert M, Dagnac T. 2010. Validation of an off line solid phase extraction liquid chromatography-tandem mass spectrometry method for the determination of systemic insecticide residues in honey and pollen samples collected in apiaries from NW Spain. *Analytica Chimica Acta* 672(1-2, Sp. Iss. SI).
- Genersch E, 2010. Honey bee pathology: current threats to honey bees and beekeeping. *Appl Microbiol Biotechnol* 87, 87-97
- Genersch E, Von der Ohe W, Kaatz H, Schroeder A, Otten C, Büchler R, Berg S, Ritter W, Mühlen W, Gisder S, Meixner M, Liebig G, Rosenkranz P 2010. The German bee monitoring

- project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie* 41, 332–352
- Girolami V, Mazzon L, Squartini A, Mori N, Marzaro M, Di Bernardo A, Greatti M, Giorio C, Tapparo A. 2009. Translocation of Neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees. *Journal of Economic Entomology* 102:1808-1815.
- [Guez D](#), [Suchail S](#), [Gauthier M](#), [Maleszka R](#), [Belzunces LP](#) (2001) Contrasting effects of imidacloprid on habituation in 7- and 8-day-old honeybees (*Apis mellifera*). *Neurobiol Learn Mem* 76: 183-191.
- Halm MP, Rortais A, Arnold G, Tasei JN, Rault S. 2006. New risk assessment approach for systemic insecticides: The case of honey bees and imidacloprid (Gaucho). *Environmental Science & Technology* 40:2448-2454.
- Higes M, Martin-Hernandez R, Martinez-Salvador A, Garrido-Bailon E, Gonzalez-Porto AV, Meana A, Bernal JL, del Nozal MJ, Bernal J. 2010. A preliminary study of the epidemiological factors related to honey bee colony loss in Spain. *Environmental Microbiology Reports* 2:243-250.
- Iwasa T, Motoyama N, Ambrose JT et al (2004) Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. *Crop Prot* 23: 371-378.
- Johnson RM, Ellis MD, Mullin CA & Frazier M 2010. Pesticides and honey bee toxicity – USA. *Apidologie* 41, 312-331
- Kadar A, Faucon JP. 2006. Determination of traces of fipronil and its metabolites in pollen by liquid chromatography with electrospray ionization-tandem mass spectrometry. *Journal of Agricultural and Food Chemistry* 54:9741-9746.
- Kluser S, Neumann P, Chauzat M-P & Pettis JS 2011. UNEP Emerging Issues: Global Honey Bee Colony Disorder and Other Threats to Insect Pollinators. www.unep.org; 12 pages
- Krischik VA, Landmark AL, Heimpel GE. 2007. Soil-applied imidacloprid is translocated to nectar and kills nectar-feeding *Anagyrus pseudococci* (Girault) (Hymenoptera : Encyrtidae). *Environmental Entomology* 36:1238-1245.
- Lambin M, Armengaud C, Raymond S, [Gauthier M](#) (2001) Imidacloprid-induced facilitation of the proboscis extension reflex habituation in the honeybee. *Arch Insect Biochem Physiol* 48: 129-134.
- Laurent FM, Rathahao E. 2003. Distribution of [C-14]imidacloprid in sunflowers (*Helianthus annuus* L.) following seed treatment. *Journal of Agricultural and Food Chemistry* 51:8005-8010.
- Li X, Bao C, Yang D, Zheng M, Li X, Tao S 2010. Toxicities of fipronil enantiomers to the honeybee *Apis mellifera* L and enantiomeric compositions of fipronil in honey plant flowers. *Environ Toxicol Chem* 29: 127-132.
- Maini S, Medrzycki P & Porrini C, 2010. The puzzle of honey bee losses: a brief review. *Bull of Insectology* 63, 153-160
- Maxim L & Van der Sluis JP 2007. Uncertainty: cause or effect of stakeholders' debates? Analysis of a case study: the risk for honeybees of the insecticide Gaucho®. *Science of the Total Environment* 376, 1-17
- Mayer DF, Lunden JD. 1999. Field and laboratory tests of the effects of fipronil on adult female bees of *Apis mellifera*, *Megachile rotundata* and *Nomia melanderi*. *J Apicult Res* 38: 191-197.
- Morandin LA & Winston ML 2003. Effects of novel pesticides on bumble bee (Hymenoptera: Apidae) colony health and foraging ability. *Environ Entomol* 32, 555-63
- Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, vanEngelsdorp D, Pettis JS. 2010. High Levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *Plos One* 5(3).
- Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, vanEngelsdorp, D & Pettis JS 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *PlosOne* 5(3), e9754. doi:10.1371

- Nauen R, Ebbinghaus-Kintscher U, Schmuck R. 2001. Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). *Pest Manag Sci* 57: 577-586.
- Neumann P & Carreck NL 2010. Honey bee colony losses. *Journal of Apicultural Research* 49, 1-6
- Nguyen BK, Saegerman C, Pirard C, Mignon J, Widart J, Thirionet B, Verheggen FJ, Berkvens D, De Pauw E & Haubruge E. 2009. Does Imidacloprid Seed-Treated Maize Have an Impact on Honey Bee Mortality? *J. Econ. Entomol.* 102(2): 616-623
- Nguyen BK, Saegerman C, Pirard C, Mignon J, Widart J, Tuirionet B, Verheggen FJ, Berkvens D, De Pauw E, Haubruge E. 2009. Does imidacloprid seed-treated maize have an impact on honey bee mortality? *Journal of Economic Entomology* 102:616-623.
- Pirard C, Widart J, Nguyen BK, Deleuze C, Heudt L, Haubruge E, De Pauw E, Focant JF. 2007. Development and validation of a multi-residue method for pesticide determination in honey using on-column liquid-liquid extraction and liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A* 1152:116-123.
- Ramirez-Romero R, Chaufaux J, Pham-Delegue MH (2005) Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee *Apis mellifera*, a comparative approach. *Apidologie* 36: 601-611.
- Rortais A, Arnold G, Halm MP, Touffet-Briens, F 2005. Modes of Honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* 36, 71-83
- Rortais A, Arnold G, Halm MP, Touffet-Briens F. 2005. Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* 36:71-83.
- Scharf ME, Siegfried BD, Meinke LJ, Chandler LD. 2000. Fipronil metabolism, oxidative sulfone formation and toxicity among organophosphate- and carbamate-resistant and susceptible western corn rootworm populations. *Pest Management Science* 56:757-766.
- Schmuck R (1999) No causal relationship between Gaucho seed dressing in sunflowers and the French bee syndrome. *Pflanzenschutz Nachrichten Bayer* 52: 257-299.
- Schmuck R, Schoning R, Stork A, Schramel O et al (2001) Risk posed to honeybees (*Apis mellifera* L. Hymenoptera) by an imidacloprid seed dressing of sunflowers. *Pest Manag Sci* 57: 225-238.
- Schmuck R, Schoning R, Stork A, Schramel O. 2001. Risk posed to honeybees (*Apis mellifera* L. Hymenoptera) by an imidacloprid seed dressing of sunflowers. *Pest Management Science* 57:225-238.
- Scott-Dupree CD, Conroy L & Harris CR 2009. Impact of currently used or potentially useful insecticides for canola agroecosystems on *Bombus impatiens*, *Megachile rotundata* and *Osmia lignaria*. *J Econ Entomol.* 102, 177-182
- Smodis Skerl MI, Velikonja Bolta S, Basa Cesnik H, Gregorc A. 2009. Residues of Pesticides in honeybee (*Apis mellifera carnica*) bee bread and in pollen loads from treated apple orchards. *Bulletin of Environmental Contamination and Toxicology* 83:374-377.
- Stark JD, Jepson PC, Mayer DF. 1995. Limitation to the use of topical toxicity data for prediction of pesticide side-effect in the field. *J Econ Entomol.* 1081-1088.
- Suchail S, De Sousa G, Rahmani R, Belzunces LP. 2004a. In vivo distribution and metabolisation of C-14-imidacloprid in different compartments of *Apis mellifera* L. *Pest Management Science* 60:1056-1062.
- Suchail S, Debrauwer L, Belzunces LP. 2004b. Metabolism of imidacloprid in *Apis mellifera*. *Pest Management Science* 60:291-296.
- Suchail S, Guez D and Belzunces LP. 2001. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environ Toxicol Chem* 20: 2482-2486.
- Suchail S, Guez D, Belzunces LP. 2000. Characteristics of imidacloprid toxicity in two *Apis mellifera* subspecies. *Environmental Toxicology and Chemistry* 19: 1901-1905.

- Tasei JN, Lerin J & Ripault G 2000. Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test. *Pest Manag Sci* 56, 784-788
- Tasei JN, Ripault G & Rivault E 2001. Hazards of imidacloprid seed coating to *Bombus terrestris* (Hymenoptera: Apidae) when applied to sunflower. *J Econ Entomol* 94, 623-627
- Thompson HM. 2010. Risk assessment for honey bees and pesticides—recent developments and ‘new issues’. *Pest Management Science* 66:1157-1162.
- Visser, A 2009. Subletale effecten van neonicotinen. *Bijennieuws* 12, juli 2009. Electronische Nieuwsbrief bijen@wur
- Visser, A 2010 Invloed van imidaclopridresiduen in oppervlaktewater op bijensterfte in Nederland. Rapport CAH Dronten opleiding Dier- en gezondheidszorg. 61 pagina's
- Von Der Ohe, W & Janke M 2009 Bienen im Stress. Schäden entstehen wenn verschiedene Faktoren zusammen kommen. *Allgemeine Deutsche ImkerZeitung* 2009/4, 10-11.
- Wu JY, Anelli CM & Sheppard WS, 2011. Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity. *PlosOne* 6 (2), e14720.
- Yang EC, Chuang YC, Chen YL & Chang LH 2008. Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *J Econ Entomol* 101, 1743-48
- Yang EC, Chuang YC, Cheng YL et al. 2008. Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *J Econ Entomol* 101: 1743-1748.