STATEMENT OF EFSA

Statement on the findings in recent studies investigating sub-lethal effects in bees of some neonicotinoids in consideration of the uses currently authorised in Europe[1]

European Food Safety Authority[2, 3]

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ABSTRACT

The European Food Safety Authority was requested to perform a comparison between the doses of several neonicotinoids tested in the studies from Henry *et al.* (honeybees, thiamethoxam) and Whitehorn *et al.*, (bumblebees, imidacloprid) published in Science (2012) with exposure of bees, following the actual use of these neonicotinoids. A third study investigating sub-lethal effects on honeybees for clothianidin and imidacloprid was also considered (Schneider *et al*., 2012). Data of uses authorised in EU and data on residues in pollen and nectar were collected to compare the actual exposure of bees with the investigated doses. The residue data were limited and available only for some crops; therefore, the extrapolation to other crops was not considered appropriate. In the studies on honeybees, the highest residue levels of thiamethoxam, clothianidin and imidacloprid in nectar were compared with the actual concentrations tested. The results indicated that the tested concentrations were higher than the concentrations found in nectar. The residue intake was estimated using different exposure scenarios. The results indicated that the doses tested in these publications were lower for clothianidin and for thiamethoxam than the estimated exposure. For imidacloprid the doses tested were higher in all the scenarios. In the studies on honeybees, the total amount of active substance was consumed by honeybees within a relatively short period instead of being not administrated over a longer period *i.e* a day. In the study on bumblebees the tested concentrations were in the range of the highest residues of imidacloprid in pollen and nectar. However, the relevance of the exposure period in the study is unknown. The comparison between the doses tested in the studies with the actual doses with the exposure of bees was considered feasible only for the seed treatment uses to maize, sunflower, oilseed rape and alfalfa. Further data would be necessary before drawing a definite conclusion on the behavioural effects regarding sub-lethal exposure of foragers exposed to actual doses of neonicotinoids

KEY WORDS

Neonicotinoids, sub-lethal effects, honeybees, bumblebees, exposure, nectar

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SUMMARY

Following a request from the European Commission, EFSA performed a comparison between the doses of several neonicotinoids tested in the studies from Henry *et al.* (honeybees, thiamethoxam) and Whitehorn *et al.,* (bumblebees, imidacloprid) (Science, 2012) with potential exposure of bees following actual use of neonicotinoids. In these studies, the authors suggested that field-realistic levels of neonicotinoids thiamethoxam and imidacloprid might have a considerable effect on colony stability and survival of honeybees and bumblebees. A third study investigating similar effects on honeybees for clothianidin and imidacloprid was also considered (Schneider *et al*., 2012).

To compare the actual exposure of bees to residues arising from the EU authorised uses with doses investigated in the published research, EFSA collected data on the products and their uses authorised in the Member States (GAP tables), as well as information on the uses considered representative for the active substance approvals (review reports). The available residue data in pollen and nectar, as provided to the Member States by the applicants, were also collected in order to define the extent of the contamination of these feed items resulting from the authorised uses.

A comparison was made between the EU representative uses, as reported in the review reports of the active substances, the uses authorised in the Member States, and the application rates investigated in the residue studies. Overall, the available residue data in nectar and pollen were limited and available only for seed treatments of maize (only pollen), oilseed rape, *Phacelia*, alfalfa and sunflower; therefore the extrapolation to crops, other than those mentioned above, was not considered appropriate.

The available highest residue levels of thiamethoxam, clothianidin and imidacloprid in nectar were compared with the actual concentrations tested on honeybees by Henry *et al.* and Schneider *et al.* and the results of the comparison indicated that the tested concentrations were higher than the concentrations found in nectar. When the residue intake was estimated using different exposure scenarios, the results indicated that the doses tested by Schneider *et al*. for imidacloprid were higher than the potential estimated exposure. However, the doses tested for clothianidin by Schneider *et al.* and for thiamethoxam by Henry *et al*. were lower than the potential estimated exposure in some scenarios. These results indicate that sub-lethal effects following the use of these active substances could not be fully excluded in worst case situations. However, it should be noted that there are several uncertainties regarding these results, therefore, they should be considered with caution. In particular, in the studies from Henry *et al* and Schneider *et al.* bees consumed the total amount of active substance within a relatively short period and not administered over a longer period *i.e*. a day. Depending on the substance properties and how fast the substance can be metabolised by the bees, this method of exposure could have lead to more severe effects than what may occur when bees are foraging.

The concentrations tested on bumblebees by Whitehorn *et al*. were in the range of the maximum exposure residues of imidacloprid in pollen and nectar. However, it is uncertain as to what extent the exposure situation in the study is representative to field conditions since bumblebees would need to forage for two weeks exclusively on imidacloprid-treated crops in order to be exposed to the same extent as in the study. Further consideration would be necessary to understand whether this situation may occur in intensive monoculture landscapes.

The results of the published studies were considered unlikely to be of relevance for other neonicotinoids (i.e. acetamiprid and thiacloprid).

Overall, before drawing definite conclusions on the behavioural effects regarding sub-lethal exposure of foragers exposed to actual doses of neonicotinoids it would be necessary to repeat the experiments performed in the studies with other exposure levels or in other situations.

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TERMS OF REFERENCE

On 3 April 2012 EFSA received a request from the European Commission for scientific and technical assistance to address issues related to the actual bee exposure following the approved uses of the neonicotinoids in Europe and the findings of two recently published papers (Henry *et al.*, 2012 and Whitehorn *et al.*, 2012), suggesting that field-realistic levels of neonicotinoids imidacloprid and thiamethoxam might have a considerable effect on bee colony survival.

In particular EFSA was requested by the European Commission to "provide a scientific statement addressing the following questions:

(1) Are the doses used in the studies referred to in the new scientific articles comparable to the actual doses which bees are exposed, based on the supported uses at EU level and on the authorisations granted by Member States?

(2) Could the new results be applied also to other neonicotinoids used for seed treatment, and in particular to clothianidin"

The agreed deadline for providing the statement is 31 May 2012.

ASSESSMENT

1. Introduction

On 30 March 2012, two papers (Henry *et al.*, and Whitehorn *et al*.) were published in 'Science' regarding the potential impact of neonicotinoids on honeybees (*Apis mellifera*) and bumblebees (*Bombus terrestris*). In these studies, it is suggested that field-realistic levels of neonicotinoids thiamethoxam and imidacloprid might have a considerable effect on bee colony stability and survival.

The article from Henry *et al.* highlights research indicating that exposure to non-lethal doses of thiamethoxam causes high mortalities in honey bees due to homing failure at levels that could put a colony at risk of collapse. The radiofrequency identification (RFID) methodology was used to estimate the homing failure. The same methodology was also used by Schneider *et al.* in another research study, published in Plos ONE 2012, where, following the investigation of sub-lethal doses of imidacloprid and clothianidin, effects on the foraging behaviour were observed.

The article from Whitehorn *et al.* indicated that colonies of bumblebees treated with imidacloprid had significantly reduced growth rate and new queen production. A detailed description and a scientific evaluation of Science papers are reported below.

The European Commission submitted to EFSA a request for scientific and technical assistance to investigate whether the actual bee exposure following the authorised uses of neonicotinoids in Europe is comparable with the exposure levels used in the research reported in the Henry *et al.* and Whitehorn *et al*.

As reported in the terms of reference, EFSA was requested to address the following questions in particular:

1) *Are the doses used in the studies referred to in the new scientific articles comparable to the actual doses to which bees are exposed, based on the supported uses at EU level and the authorisations granted by Members States?*

2) *Could the new results be applied also to other neonicotinoids used for seed treatment, and in particular to clothianidin?*

To answer to the question 1 and the first part of the question 2 (*Could the new results be applied also to other neonicotinoids*) EFSA gathered data from the Member State competent authorities on the approved uses (i.e. GAP tables) and on the residues in pollen and nectar for thiamethoxam, imidacloprid, clothianidin, acetamiprid and thiacloprid, in the framework of both the EU and national regulatory processes. Bee toxicity endpoints (*i.e.* acute LD_{50} values from standard laboratory studies) of the neonicotinoids were also considered. To answer to the second part of question 2 (*and in particular to clothianidin*), the study from Schneider *et al*. was considered.

1.1. Summary and evaluation of the new published research

1.1.1. Summary of Henry et al.

In this experiment the homing behaviour of individual bees exposed to the neonicotinoid thiamethoxam was investigated. Individual bees were exposed to a dose of 1.34 ng thiamethoxam in a 20µL sucrose solution (67 ppb). Control (unexposed, n=72) bees and treated bees (n=74) from the same colony were released in the first experiment 1 km away from the colony at a site the bees foraged before the experiment. In the second experiment bees from other colonies (n=118 in controls and treatments) were released 1 km away as well but at random sites where bees did not forage immediately before the start of the experiment. The test design aimed at assessing different situations. In experiment 1 of the study it was expected that navigation is easier because bees were familiar with the way back to the hive whereas in experiment two bees were unfamiliar with the way back to the hive and as a result navigation back to the colony would be more difficult. Experiment 1 was repeated in experiment 3 but with release of bees (unexposed, n=6;, treated, n=67) at a shorter distance from the hive (only 70 m instead of 1 km). The same test as in experiment 2 was conducted in experiment 4 but in a different landscape (suburban landscape instead of agricultural landscape) with 82 exposed and 54 unexposed bees.

The radiofrequency identification (RFID) method was used to follow individual bees as they entered and exited from colonies. Bees were marked with a unique radio frequency microchip and recorded when entering the hive equipped with automatic readers. Effects of treatment with thiamethoxam on the cumulative probability to return to the hive were observed. Homing failure was estimated as the proportion of non-returning treated foragers relative to the proportion of returned control foragers and the study authors equated this value to mortality. The results of the experiment 1 and 2 indicated that 10.2% to 31.6% of the thiamethoxam exposed bees respectively, failed to return to the hive. Experiments 3 and 4 resulted in lower homing failure rates of 6.1% and 9.8%, respectively.

The homing failure rates of the first two experiments were used in a population dynamic model in order to simulate the potential impact on the colony survival. The model was run with egg laying rates of queens of 2000, 1800 and 1600 per day. During the time of modelled exposure of foragers (30 days) the total number of bees in the colony decreased followed by recovery of the colony and increasing number of bees in the colony as soon as exposure stopped. With an egg-laying rate of 1600 per day the colony declined even without treatment-related effects.

An experimental design using the radiofrequency identification (RFID) method to monitor the influence of sub-lethal doses of insecticides on individual honeybee foragers on an automated basis, was also used by Schneider *et al*. With electronic readers positioned at the hive entrance and at an artificial food source, they obtained quantifiable data on honeybee foraging behaviour. Several groups of bees were compared, fed simultaneously with different dosages of imidacloprid (0.15–6 ng/bee) and clothianidin (0.05–2 ng/bee). Both substances led to a significant reduction of foraging activity and to longer foraging flights at doses of ≥0.5 ng/bee (clothianidin) and ≥1.5 ng/bee (imidacloprid) during the first three hours after treatment. The distance between the hive and the feeder in this study was 7 m.

1.1.2. Evaluation of Henry et al.

The study from Henry *et al*. is interesting since it applies a relatively new technology (i.e., the use of RFID on honeybees) to monitor homing behaviour of individual bees after sub-lethal exposure to an insecticide. The method could significantly improve future test designs of field studies and reduce observational bias.

One of the key points is whether the exposure level evaluated in the study was comparable to field situations. The exposure estimate was based on calculations of sugar uptake of foragers and residue data which were available for France. An average residue value of 1.85 µg thiamethoxam/kg nectar was used to calculate an equivalent dose of 1.34 ng thiamethoxam/bee. Individual bees were dosed with 1.34 ng in a 20 µL sugar solution.

The model which was used to calculate the exposure is based on the daily sugar uptake of bees (see Rortais *et al*. 2005). The consumption model was considered realistic (EFSA, 2012) and it was used in the present statement (see paragraph 3.5.2). The average residue value of 1.85 µg thiamethoxam/kg nectar is also in a realistic range but not the worst-case residue value as reported in **Table 6**. The maximum residues exceed this residue value by a factor of 2.8.

The exposure conditions in the experiment constitute a potential worst case scenario. The exposure of 1.34 ng thiamethoxam/bee is based on the daily exposure of a honey bee foraging the whole day. But in the experiment, bees consumed the total amount of active substance (*i.e*. 1.34 ng) within a relatively short period of time (after a 90 minute starvation period) and not distributed over the entire day. Depending on the substance properties and how fast the substance can be metabolized by the bees, this method of exposure could have lead to more severe effects than what may occur when bees are dosed with 1.34 ng distributed over a longer duration, *i.e.*, a whole day of foraging.

Individual bees were obtained from 3 different colonies for the different experiments (1+2, 3 and 4). This might explain why less severe effects were observed in the experiments No. 3 and 4. This highlights the need for replication at the colony level to account for such variations. The importance of ability to return from surroundings where bees did not forage immediately before the start of the experiment also requires evaluation.

The modelling reported in the study suggested that as soon as exposure of the foragers is reduced below that observed to result in forager losses, the colonies will recover (for assumed egg-laying rates of 2000 and 1800 eggs per day).

Overall it is concluded that the study is very interesting in terms of the test methods, *i.e.*, the use of RFID to track bee movement and in terms of the test results, considered in the context of population modeling estimates. The study raises important issues such as the impact of sub-lethal effects on colony survival. However the exposure in the test seems to be too severe compared to real field situations. The experiment was repeated with bees unfamiliar with the way back to the hive in a suburban environment. Under these conditions the effects on homing behaviour were much less pronounced compared to experiment 2 where bees were unfamiliar with their release sites; however sites were located in a more rural environment. Before drawing definite conclusions on the behavioural effects regarding sub-lethal exposure of foragers and the consequences to the colony it would be necessary to repeat the study with other substances and with more realistic exposure levels in order to see whether similar results would be obtained.

1.1.3. Summary of Whitehorn et al.

In this study the effects of the neonicotinoid imidacloprid on the weight gain and production of queens of the bumblebee species *Bombus terrestris* were investigated. Colonies were kept in the laboratory for two weeks and fed pollen and sugar solution *ad libitum*. The low treatment group was exposed to pollen and sugar water containing 6 µg imidacloprid/kg and 0.7 µg imidacloprid/kg, respectively, and the high treatment group was exposed to pollen and sugar water containing 12 µg imidacloprid/kg and 1.4 µg imidacloprid/kg, respectively. Controls were provided untreated pollen and sugar water. After two weeks in the laboratory the colonies were placed in the field and their development was monitored for 6 weeks. After the 6 weeks post-treatment period, the production of queens was significantly less in the imidacloprid-treated colonies. The mean number of queens was 13.72 in control colonies compared to only 2 and 1.4 in the low and high treatment groups, respectively. Also the weight gain of treated colonies was reduced compared to controls. At the end of the study, colonies in the low and high treatment group were respectively 8% and 12% smaller than controls.

1.1.4. Evaluation of Whitehorn et al.

The study investigates effects of sub-lethal exposure of bumblebees to imidacloprid. Effects on reproduction were observed under the tested conditions. The concentrations used in the study were in the range of the maximum exposure residues of imidacloprid in pollen/nectar found in the EU (see **Table 6**). The exposure in the test during the two weeks in the laboratory was a worst case exposure scenario since bumblebees could only feed on imidacloprid spiked pollen and sugar water. It is uncertain as to what extent such an exposure situation is representative of field conditions since bumblebees would need to forage for two weeks exclusively on imidacloprid-treated crops in order to be exposed to the same extent as in the study. Winter oilseed rape crops flower for around 3-4 weeks. Further data on foraging behaviour (*e.g.*, extent of crop fidelity) of bumblebees would be needed in order to address this question.

The authors hypothesize that there is a direct link between the observed reduction in weight gain of the colonies and the reduced production of queens. This was not investigated in the Whitehorn *et al*. study but is rather based on a field study by Müller and Schmid-Hempel (1992) with the species *B. lucorum*.

The authors link the effect on reduced weight gain of colonies to reduced foraging efficiency of exposed bees; however, foraging efficiency was not investigated in the study. A reference is made to two other studies, *i.e.*, Mommaerts *et al*. (2010) and Ramirez-Romero *et al*. (2005) where effects of imidacloprid on learning and foraging behaviour were studied.

Nevertheless a clear effect on queen production where mean number of queens (reproductives) was significantly lower ($p<0.008$) in the low (mean=2.00) and high (mean=1.4) imidacloprid treatment groups compared to the controls (mean=13.7) was observed. Such an effect could also be a direct reproductive effect and not necessarily the consequence of reduced foraging.

Based on the results of their study and residues reported in pollen from other studies, the authors predict widespread and significant impacts of imidacloprid on bumble bee colony reproduction. However, the conclusions regarding the widespread impact of imidacloprid on bumblebee reproduction would need to be investigated further. For example it should be clarified whether there are sufficient monitoring data available to draw a conclusion on whether bumblebee species, *e.g*., *B. terrestris*, inhabiting agricultural landscapes are indeed in decline.

2. Material and methods

2.1. Data on approved uses

Data on the authorised uses of imidacloprid, thiamethoxam, clothianidin, acetamiprid and thiacloprid were collected from Member States (MSs) and were compiled in an Excel© spreadsheet. These data were considered useful to get an overview of all the uses authorised. The representative uses evaluated for the approval of the active substances at EU level were also included in the Excel© spreadsheet. Data were collected from the review reports established as a result of the evaluation of the active substances (European Commission, 2004a, 2004b, 2005, 2006, 2008). It is important to note that the review reports only included the uses for which the risk assessment was considered completed and not all the representative uses originally proposed by applicants and evaluated at EU level.

When available, the following information was included in the database: formulated product name; Member State where authorised; crop; field or greenhouse use; method of application, number of applications and growth stage; minimum and maximum application rates in g a.s./ha. However, the GAP tables provided were not harmonised in terms of product names, type of applications and application rates. Furthermore, some Member States did not submit information, or the data submitted were in a format that could not be processed. Therefore, this data set should not be considered as exhaustive.

As regards the data from Members States, the percentage in terms of number of uses authorised per Member States, per individual active substance, per method of application were calculated. Due to the limitation of the data set, all the analyses carried out should be considered only as a preliminary assessment.

2.2. Data on residue in nectar and pollen

Numerous studies on bees for imidacloprid, thiamethoxam, clothianidin, acetamiprid and thiacloprid were provided by the Member States. Studies investigating residues in nectar and pollen were selected and an Excel© spreadsheet was set up. When available, the following information was included in the spreadsheet: formulated product name; dose (in terms of g a.s/ha); seed dressing rate (nominal and analytical finding); seed drilling rate, use type and crop; test type, country and GLP compliance; minimum and maximum residue (mg a.s/kg) in pollen and nectar; limit of quantification (LOQ, mg/kg) and limit of detection (LOD, mg/kg). Some Member States did not submit information and a comprehensive reference list was not available. Therefore, this data set cannot be considered as exhaustive.

2.3. Comparison of residue data *vs* approved uses

The key parameters of the residue studies were analysed and compared with the representative EU uses and the Member States' GAPs. The parameters for the comparison, over the mode of treatment, were the application rate (seed dressing rate and/or mass of active ingredient per hectare) and the location where the study was conducted. Regarding the location, all the studies conducted in Europe were deemed to be suitable without investigating details such as soil, weather or agricultural conditions. These conditions of a study conducted in North America (USA and Canada) were however compared with European conditions to judge on the representativeness of this study to EU. This analysis was not done for a study conducted in Argentina since the results of these study (no residues detected) were not used further.

2.4. Comparison of the tested doses with the actual exposure

The evaluation was focused on the oral route of exposure. Other potential routes of exposure such as contact, inhalation, or consumption of guttation water were not considered in the context of this statement. The highest residue data on nectar were compared with the doses tested in Henry *et al.* and Schneider *et al.* studies because only oral treatments in sugar solution were administered to the tested honeybees. It is noted that the oral exposure via consumption of nectar for honeybee foragers is considered the most relevant, while the oral exposure via pollen is reported to be not relevant in EFSA (2012).

Two approaches were followed to compare the residue values with the doses tested on honeybees by Henry *et al*. and Schneider *et al*.: 1) a first comparison was carried out between the concentrations of sugar solutions used in the papers and the residue data in nectar; 2) a second comparison considered the doses used in the papers and the estimated residue intake via consumption of contaminated nectar.

For the comparison with Whitehorn *et al.* both residue data on nectar and pollen were considered. Since the doses tested by Whitehorn *et al.* were reported in µg/kg a direct comparison of theses doses with residue data was performed.

2.5. Applicability of the published results to other neonicotinoids

Toxicity data from the review reports and from the Draft Assessment Reports (Belgium, 2003; Germany, 2005; Spain, 2002) were collected. These endpoints represent the worst case values (i.e. the lowest values) available in the dossiers evaluated for the approval of the active substances. Along with the data on uses and on residue, the toxicity endpoints were considered useful to address whether the results of the published paper can be applied to other neonicotinoids (i.e. acetamiprid and thiacloprid) used as seed treatment.

3. Results and discussion

3.1. Data on uses from Member States (GAP tables)

Of the number of uses authorised in Member States (representing more than 1000 uses), more than 15% are in Germany and Spain, and more than 10% are in UK, Italy and the Netherlands. The overall view is reported in **Figure 1**.

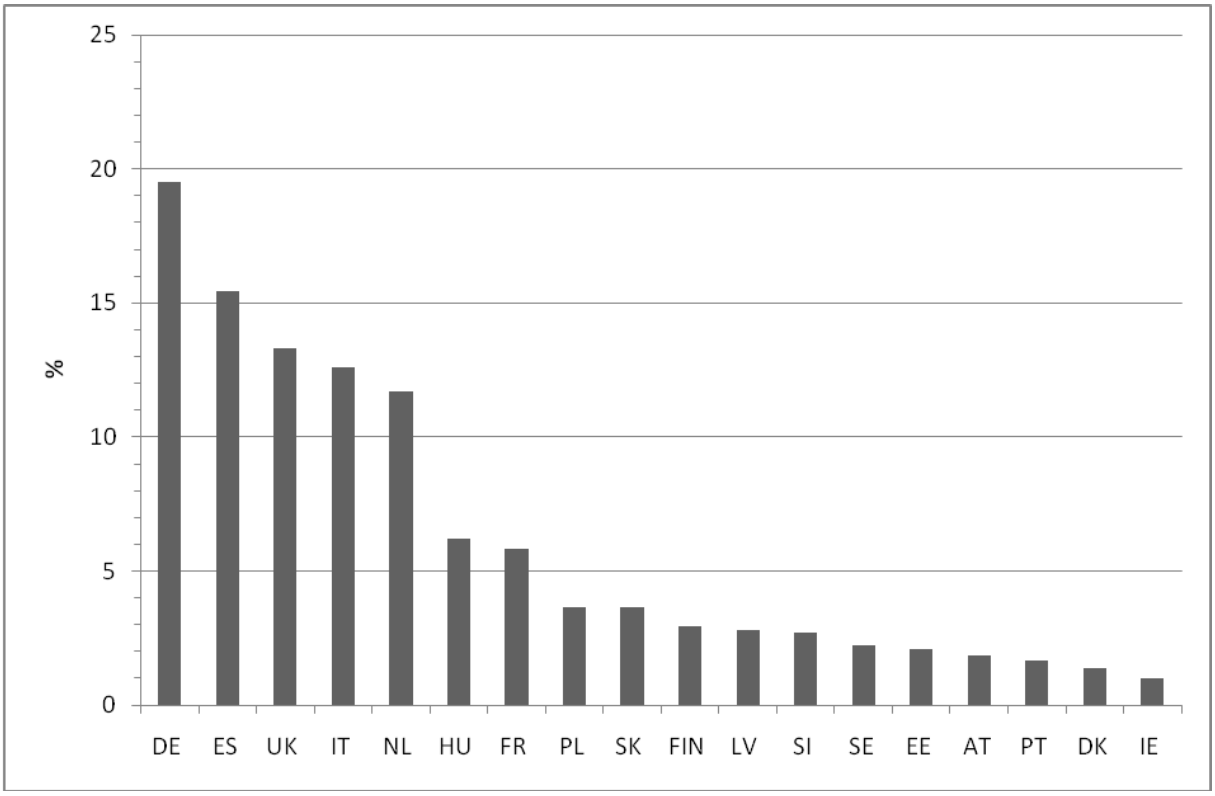

Figure 1: Percentage of number of neonicotinoid uses authorised in the Member States (source data: GAP tables provided by MSs)

In terms of individual active substances, imidacloprid and thiamethoxam account for the largest percentages of authorised uses with more than 30% and 25%, respectively. Thiacloprid and acetamiprid account for more than 15%, while clothianidin accounts for less than 5% of the authorised uses (**Figure 2**). These uses represent more than 200 products. They include field, greenhouse and indoor uses with the field uses representing the vast majority (>60%; **Figure 3**).

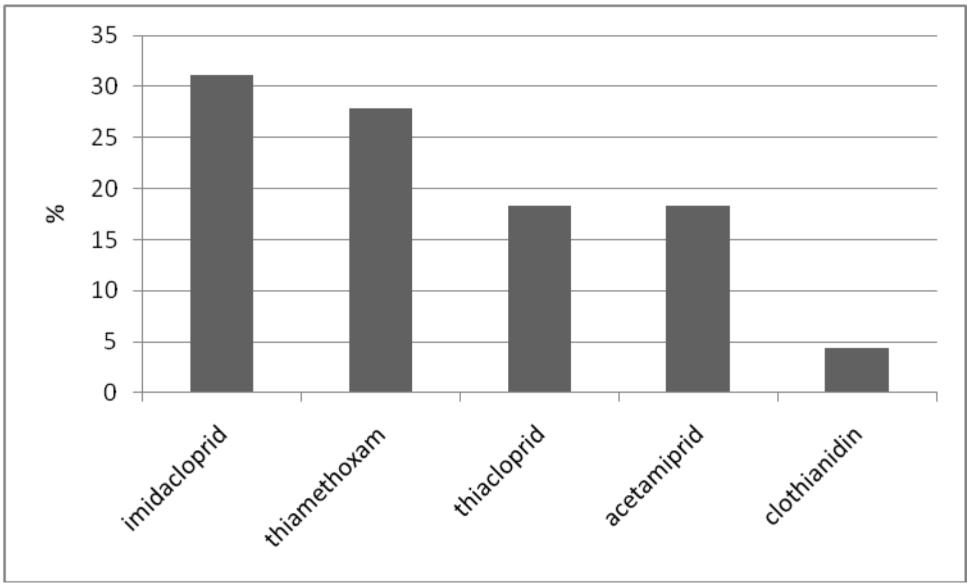

Figure 2: Percentage of number of uses per active substance authorised in the Member States (source data: GAP tables provided by MSs)

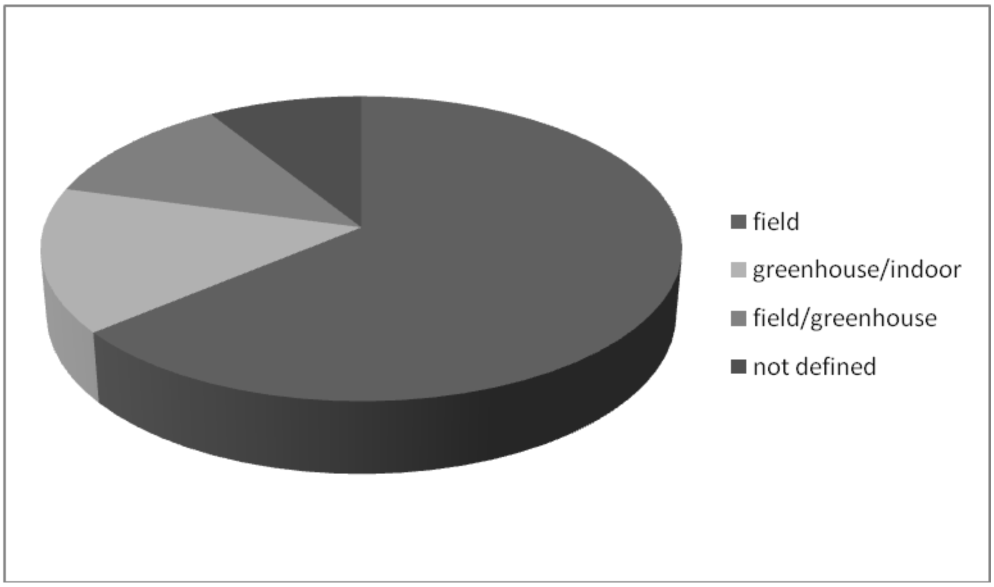

Figure 3: Overview of the situations of uses authorised in the Member States (source data: GAP tables provided by MSs)

Approximately 70% of the field uses are applied by spraying, while less than 20% are seed treatment and less than 20% are other methods of application such as drip irrigation, soil disinfectant, *etc*. (**Figure 4**).

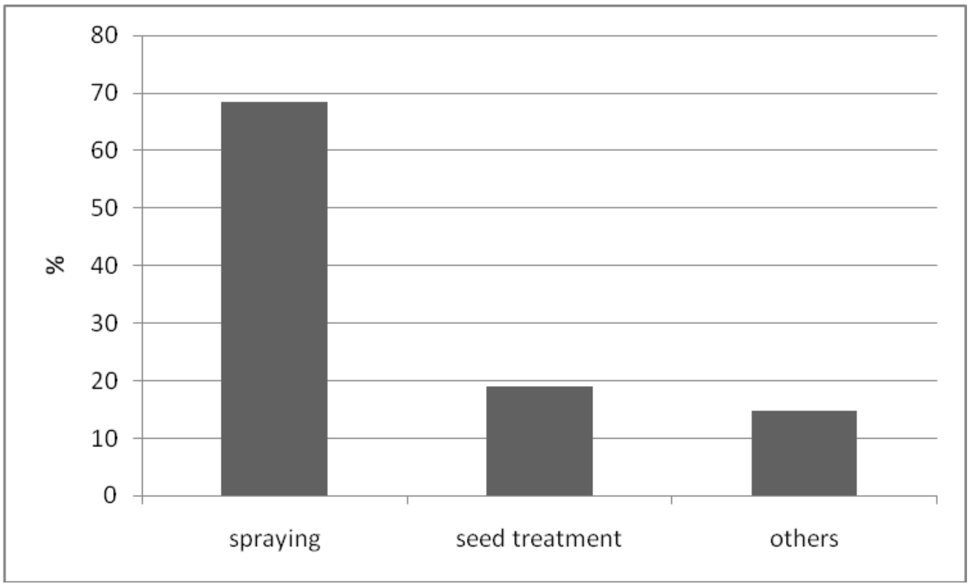

Figure 4: Overview of the methods of application within the authorised uses (source data: GAP tables provided by MSs)

The percentage of the uses authorised for each active substance per method of application is reported in **Figure 5**. Thiacloprid and acetamiprid are authorised in Member States as spraying or soil treatment. No uses as seed treatment were noted for acetamiprid, and a single use was noted for thiacloprid (maize).

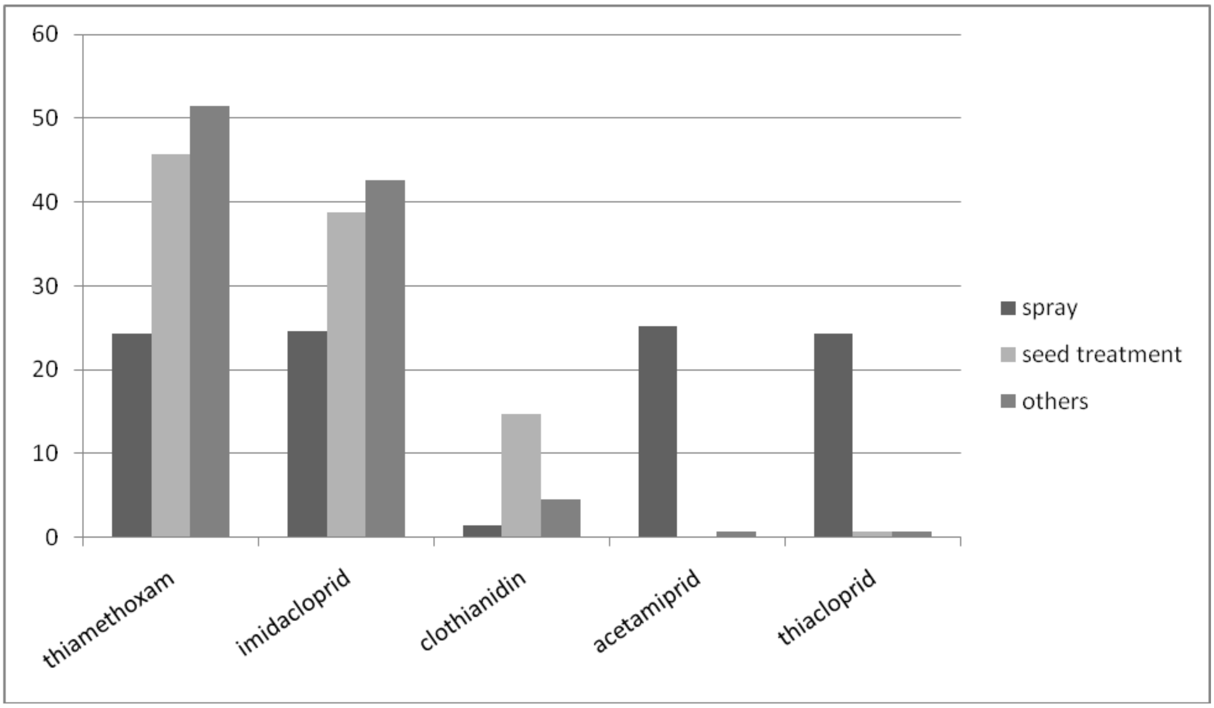

Figure 5: Overview of the number (%) uses authorised for each active substance per method of application (source data: GAP tables provided by MSs)

An overview of the crops where spray applications, seed treatments and other uses are authorised is reported in **Table 1, 2, 3**. The growth stage is highly variable.

The maximum application rates from individual active substances in relation to the spray field uses are as follows: acetamiprid 250 g a.s./ha (apple, DK); imidacloprid 350 g a.s./ha (potatoes, HU); thiamethoxam and clothianidin 150 g a.s./ha (citrus; ES and apple/pear, FR respectively); thiacloprid 360 g a.s/ha (ornamentals, IT). For the seed treatments the application rates were reported as g a.s./ha, seed dressing rate, drilling rate.

Table 1: Summary of spray uses authorised in Member States (source data: GAP tables provided by MS)

thiamethoxam	potato, nut trees, pome fruit, stone fruit, cucurbits, brassicas, citrus fruits, cotton, vines, salad, herbs, ornamentals, peppers, tobacco, tomato, floriculture crops, tree nursery, flower bulbs, cereals, carrot, sunflower, onions, oilseed rape, cotton,
clothianidin	potato, stone fruit, pome fruit
imidacloprid	ornamentals, potato, pome fruit, hops, vines, stone fruit, tobacco, pepper, flower bulbs, floriculture crops, tree nursery, stone fruit, tomato, almonds, cucurbits, artichoke, beans, brassicas, celery, citrus fruits, hazel, olives, salad, palm trees, peppers, forestry, alfalfa, cereals, strawberry.
acetamiprid	pome fruit, ornamentals, oilseed rape, turnip rape, salad, herbs, stone fruit, maize, potato, tobacco, brassicas, forestry, cucurbits, soft fruits, tomato, peppers, floriculture crops, tree nursery, flower bulbs, citrus fruits, fig, artichoke, clover, lucerne, cotton, strawberry, citrus fruits
thiacloprid	stone fruit, pome fruit, strawberry, oilseed rape, potatoes, cereals, mustard, ornamentals, soft fruits, salad, herbs, nut trees, fennel, asparagus, carrot, brassicas, celeriac, celery, onions, cucurbits, leeks, garlic, shallot, flower bulbs, beans, ornamentals, floriculture crops, tree nursery, sugar beet, fodder beet, hemp, strawberries, sunflower, maize, cotton, alfalfa, olive trees, fig trees,

Table 2: Summary of seed treatment uses authorised in Member States (source data: GAP tables provided by MS)

thiamethoxam	beet seeds, cabbage, cauliflower, cotton, kale, lettuce, maize, mustard, peas, potato, oilseed rape, sorghum, sunflower, wheat, barley, rye, oat, triticale
clothianidin	beet seeds, cereals (wheat, barley, oat, rye, triticale), maize, oilseed rape, potato, sunflower
imidacloprid	beet seeds, oat, asparagus, broccoli, cauliflower, barley, bulb crops, corn, lettuce, cabbage, brassicas, hop, leek, linseed, maize, onion, peas, potato, pumpkin seeds, oilseed rape, sunflower, wheat
thiacloprid	maize

Table 3: Summary of other uses authorised in Member States (source data: GAP tables provided by MS)

thiamethoxam	cucurbits, beans, brassicas, citrus fruit, salad, herbs, ornamentals, palm trees, peppers, tomato, flower bulbs, potato, house plants, pome fruit, forestry, citrus fruits, peppers,
clothianidin	maize, potato, sorghum, sorghum, poppy
imidacloprid	grassland, hops, salad, herbs, potato, brassicas, chicory–roots, beans, citrus fruits, cucurbits (eggplant, melons, cucumber), palm tree, peppers, tomato, rice, forestry, pome fruit, stone fruit, artichokes, vines, alfalfa, tobacco, olive trees, ornamentals, strawberries, hops,
acetamiprid	non fruiting trees and bushes
thiacloprid	ornamentals, tree nursery

3.2. Data on uses from EU review reports

An overview of uses from the review reports is shown in **Table 4** and in **Table 5** for the seed treatment and non-seed treatment uses, respectively. For clothianidin only the EU representative uses as seed treatments were reported, while for imidacloprid, acetamiprid and thiacloprid, the EU representative uses as foliar spraying were reported (only greenhouse for imidacloprid). For thiamethoxam both seed treatment and spray uses were reported.

Table 4: Summary of the EU representative uses from the review reports– seed treatments

Active substance	crop	Type of use	Max application rate (g a.s/ha)
thiamethoxam	wheat	seed treatment	91
	barley	seed treatment	105
	sunflower	seed treatment	24.5
	maize	seed treatment	73
	oilseed rape	seed treatment	34
	sugar beet	seed treatment	78
	peas	seed treatment	105
	cotton	seed treatment	63
	potato	seed treatment	135
clothianidin	sugar beet, fodder beet	seed treatment	78
	maize	seed treatment	50

Table 5: Summary of the EU representative uses from the review reports– non-seed treatments

Active substance	crop	Type of use	Max application rate (g a.s/ha)
thiamethoxam	pome fruit	foliar spraying	100
	citrus	foliar spraying	150
	cotton	foliar spraying	50
	fruiting vegetables	foliar spraying	100
	lettuce	foliar spraying	50
	melon and water melon	foliar spraying	100
	ornamentals/tomato	foliar spraying	100
	ornamentals	foliar dipping	100
	potato	foliar spraying	20
	peach	foliar spraying	50
imidacloprid	tomato[(a)]	foliar spraying	150
acetamiprid	citrus fruit	foliar spraying	100
	pome fruit	foliar spraying	75
	stone fruit	foliar spraying	75
	fruiting vegetables	foliar spraying	90
	oil seed	foliar spraying	75
thiacloprid	apple/pear	spray	180
	tomato/pepper/cucumber/orna mentals	spray	216
	ornamentals	spray	216
	peach/apricot	spray	14.4
	melon/watermelon	spray	14.4

(a): only greenhouse use

3.3. Residue data in nectar and pollen

Several nectar and pollen residue studies were available for thiamethoxam, imidacloprid, clothianidin and a single study was available for thiacloprid. With a single exception, these residue data originate from open field studies (field or semi-field studies). Data were limited to seed dressing and soil treatments. Residue measurements were performed in pollen and nectar of treated seed plants, untreated seed plants grown in pre-treated soil, and treated seed plants grown in pre-treated soil.

Data were available for the following crops: oilseed rape, maize, sunflower for imidacloprid; maize, oilseed rape, alfalfa, sunflower and *Phacelia tanacetifolia* for thiamethoxam and its metabolite CGA322704 (i.e clothianidin); maize, oilseed rape and sunflower for clothianidin and its metabolites; maize for thiacloprid and its metabolite. The majority of these studies were conducted in Europe (Germany, France, UK, Sweden). No residue data were available for acetamiprid.

In some studies only the seeds of previous crop(s) were treated and/or the soil was spiked to form a certain soil residue level before the analysed crop was seeded. These studies could be considered relevant to be compared with soil treatment uses. However, the doses used for the pre-treatment of the soils were lower than the authorised soil treatment uses and, therefore were considered likely to lead to lower soil concentrations. Therefore these studies were not used to estimate the potential exposure of bees from the soil treatment uses.

From the available data collected from Member States, the highest residue levels in nectar were clothianidin 0.0054 mg/kg (LOQ =0.001 mg/kg) in oilseed rape; thiamethoxam 0.0052 mg/kg (LOQ =0.0005 mg/kg) in *Phacelia*; imidacloprid was not detected (LOQ=0.0003 mg/kg). The highest values for pollen were 0.002 mg/kg imidacloprid (oilseed rape); 0.0114 mg/kg clothianidin (maize); 0.051 mg/kg thiamethoxam (alfalfa), the LOQ was 0.001 mg/kg. An overview of the residue data collected is reported in **Table 6**.

In the DAR for clothianidin cage tests with residue data were briefly summarized: treated oilseed rape seeds were tested in Sweden, France and Great Britain. Residue analyses were conducted, which resulted in maximum 0.0086 mg a.s./kg oilseed rape nectar and maximum 0.0017 mg a.s./kg oilseed rape pollen.

In the DAR for imidacloprid a residue value of 0.00081 mg/kg in nectar of oilseed rape and 0.0076 mg/kg in pollen of oilseed rape was reported. These values originate from the USA. The results from these studies were evaluated to be representative considering soil and meteorological conditions of the trials. The vegetation period for the summer canola, was however later than the typical periods in Europe.

A greenhouse study indicated residues of 0.0019 mg/kg in nectar and 0.0033 mg/kg in pollen of sunflower.

3.4. Comparison of residue data *vs* approved uses

Seed treatment uses

The relevant conditions of almost all of these studies were considered to be comparable with the conditions of the uses of neonicotinoids in Europe as reported in the GAPs for the same crop i.e. oilseed rape, maize, sunflower and alfalfa. However, it was noted that the application rates of some uses for imidacloprid were higher than the rates used in the residue trials with maize and sunflower.

It is important to note that residue data were only available for the above mentioned crops, however, neonicotinoids are authorised for use as a seed treatment for many other crops in Europe (**Table 2**). Therefore, without supporting data, there is uncertainty regarding the extrapolation of residue data from one crop to others.

Non-seed treatment uses

No residue data were available for any other uses than seed dressing (or soil treatment). The contamination of the plants with the active substances is basically different for spray uses compared to seed treatments, therefore the translocation and formation of residues in bee-relevant matrixes will likely be different. Without relevant supporting data, it was not possible to extrapolate the available residue data to spray uses. In the EFSA (2012) it was reported that residues in nectar or pollen following spray applications on flowering crops can be expected to be higher than those following systemic translocation.

Also the extrapolation of residue data obtained for seed treatment uses to other uses (an aerial pulverisation authorised for imidacloprid on rice, non-professional uses) was not possible. However the exposure to bees from these uses could be considered limited in time and space.

A simple comparison with the seed treatment uses, only based on the treatment rates and number of applications, was made for in-soil uses (*e.g.* soil incorporation, dipping of seedlings, drip irrigation or drenching, furrow application). Generally, it was considered that the application rates of these uses are similar (at least not appreciably higher), than those of the seed treatment uses (expressed in mass per hectare). Based on that, it is considered that in-soil uses will not lead to higher exposure to honeybees than the seed treatment uses. As an exception, it is noted that the application rates and/or the number of applications of some field uses like drip irrigation of imidacloprid in some vegetables, citrus or tobacco are much higher or more compared to the seed dressing uses. The same might be concluded for thiamethoxam used in some vegetables and ornamental bushes.

Table 6: Overview of data with the highest residue values found in nectar and pollen

Active ingredient	Crop	Max residue in nectar (mg/kg)	Max residue in pollen (mg/kg)	No. of data (nectar, pollen)	Compliance with MSs GAP[(f)]	Seed treatment[(g)]	Pre-treatment(s)
thiamethoxam (TMX)	maize	-	0.004	-, 3	yes	yes	-
	maize	-	0.012	-, 4	yes	yes	previous crop
	maize	-	<0.001	-, 2	yes	no	previous crop
	oilseed rape	0.0014	0.004	4, 5	yes	yes	-
	oilseed rape	0.0046	0.006	3, 4	yes	yes	previous crop
	oilseed rape	0.0022	0.008	2, 2	yes	no	previous crops
	phacelia	0.0052	0.039	3, 3	yes	no	previous crops
	alfalfa	0.0022	0.051	3, 2	yes	no	previous crops
	sunflower	<0.001	0.0014	6, 2	yes	no	previous crop
CGA322704 metabolite of TMX = clothianidin[(a)]:	maize	-	0.003	-, 3	yes	yes	-
	maize	-	0.007	-, 4	yes	yes	previous crop
	maize	-	<0.001	-, 2	yes	no	previous crop
	oilseed rape	<0.001	<0.001	4, 5	yes	yes	-
	oilseed rape	0.001	0.002	3, 4	yes	yes	previous crop
	oilseed rape	n.d.	0.003	2, 2	yes	no	previous crops
	phacelia	0.0023	0.004	3, 3	yes	no	previous crops
	alfalfa	0.0011	0.002	3, 2	yes	no	previous crops
	sunflower	<0.001	0.001	6, 2	yes	no	previous crop
clothianidin	maize	-	0.0114	-, 11	yes	yes	-
	maize	-	0.0019	-, 2	yes	yes	soil
	maize	-	0.0013	-, 2	yes	no	soil
	oilseed rape	0.0054	0.0025	4, 4	yes	yes	-
	oilseed rape	0.0022	0.004	1, 1	no	yes	soil
	oilseed rape	<0.001	0.001	2, 1	yes	no	soil
	oilseed rape[(b)]	0.0086	-	-	-	-	-
	sunflower	n.d.	0.0031	2, 2	yes	yes	-
imidacloprid	maize	-	n.d.	-, 4	yes	yes	-
	sunflower[(c)]	n.d.	n.d.	2, 2	no	yes	-
	oilseed rape[(d, e)]	0.00081 - <0.01	0.0076-<0.01	11, 8	yes	yes	-
	oilseed rape	<0.0003	0.002	2, 2	yes	no	previous crop and soil
thiacloprid and its metabolite KKO 2254	maize	-	<0.001	-, 1	yes	yes	-

(a): All treatments were done with the parent thiamethoxam;

(b): In the DAR of clothianidin cage tests with residue data were briefly summarized: treated oilseed rape seeds were tested in Sweden, France and Great Britain at the intended use rate. Residue analyses were conducted, which resulted in a maximum of 8.6 µg a.s./kg oilseed rape nectar sampled by bees and maximum 1.7 µg a.s./kg oilseed rape pollen sampled by bees;

(c): A 3rd field study was conducted in Argentina, but no residues were detected. A greenhouse study indicated residues of 0.0019 mg/kg in nectar and 0.0033 mg/kg in pollen;

(d): Data set includes trials from USA and Canada. The results from these studies were evaluated to be representative considering soil and meteorological conditions of the trials. The vegetation period for the summer canola, however was later than the typical periods in Europe;

(e): The studies used generally higher treatment rates than those used in EU.

(f): based on qualitative assessment of the relevant conditions of residue studies *vs* Member States GAPs;

(g):"no" means that untreated flowering crops were analysed, but they grew in soils where seed treated crops where planted the previous year or the soil was treated;

n.d: not detected;

3.5. Comparison of the tested doses to honeybees and bumblebees with the actual exposure

The highest residue levels found in nectar were considered i.e. 0.0052 mg/kg for thiamethoxam (*Phacelia*), 0.0086 mg/kg for clothianidin (oilseed rape) and 0.00081 mg/kg for imidacloprid (oilseed rape) as reported in paragraph 3.3. The doses and the methods of treatments of the published studies are summarised in the following table (**Table 7**).

Table 7: The treatment regimes in the studies from the new scientific articles

Study	Specie	Active substance	Dose(s)	Treatment(s)
Henry *et al.*	honeybee	thiamethoxam	1.34 ng/bee	single oral treatment via sucrose solution a few hours before the first observation [(a)]
Schneider *et al.*	honeybee	imidacloprid	series of 0.15, 1.5, 3.0 and 6.0 ng/bee	single oral treatment via sucrose solution a few hours before the first observation [(b)]
		clothianidin	series of 0.05, 0.5, 1.0 and 2.0 ng/bee	
Whitehorn *et al.*	bumble bee	imidacloprid	6 µg/kg pollen and 0.7 µg/kg sugar solution	*ad libitum* oral treatment via sucrose solution over 14 days before the observations
			12 µg/kg pollen and 1.4 µg/kg sugar solution	

(a): considering the time of consumption of sucrose solution, the following procedure of tagging and the additional 40 minutes before release; (b): considering the time of consumption of sucrose solution and the additional 20 minutes before release

3.5.1. *Honeybees, comparison of the sugar solution concentrations with the residues in nectar*

Based on a density of 1.23 kg/L (the density of 50 % sugar solution, as reported in Cell Biology Laboratory Manual by Heidcamp, 1995), the doses tested in the papers were calculated as µL and the residue data were expressed in µL. The active substance content in 20 µL sugar solution or 10 µL nectar was then calculated. The density of 1.23 kg/L was used as constant, although, the density of a 2M sucrose solution, used in the study by Schneider *et al*. could have been slightly higher. The results of these calculations are reported in **Table 8**.

Table 8: Comparison of concentrations and doses used in the studies by Henry *et al*. and Schneider *et al.* with highest residue levels

Active substances	Concentration used in the test	Highest concentration in nectar	Factor (test concentration /residue)	Dose used in the test	Potential dose from nectar	Factor (test dose/ residue
thiamethoxam (Henry *et al*.)	67 µg/L ~ 0.0545 mg/kg	0.0052 mg/kg	~10.5 ~8.9[(c)]	1.34 ng/bee in 20 µL sucrose solution	0.128 ng in 20 µL nectar	~10.5
clothianidin (Schneider *et al*.)	50 µg/L[(a)] ~ 0.0407 mg/kg[(d)]	0.0086 mg/kg	~4.7	0.5 ng/bee[(a)] in 10 µL sucrose solution	0.106 ng in 10 µL nectar	~4.7
imidacloprid (Schneider *et al*.)	150 µg/L[(a)] ~ 0.122 mg/kg[(d)]	0.00081 mg/kg[(b)]	~150	1.5 ng/bee[(a)] in 10 µL sucrose solution	0.01 ng in 10 µL	~150

(a) the second dose from the range of doses, at which dose level sub-lethal effects were described by the authors;
(b) the majority of the available analytical measurements used relatively high detection limits, therefore the uncertainty of the representativeness of this value is higher than for the others;
(c) factor calculated for the sum of maximum residues of thiamethoxam and its metabolite clothianidin;
(d) reported in the studies as 38 µg/kg and 115 µg/kg;

The concentrations tested in the papers from Henry *et al*. and Schneider *et al*. appear to be higher than the highest residue levels in nectar. In particular a factor of approximately 10.5X was estimated with respect to the dose tested by Henry *et al*. for thiamethoxam, and a factor of approximately 4.7X and 150X with respect to the doses tested by Schneider *et al*. for clothianidin and imidacloprid, respectively.

This comparison suggests that the authors used higher doses in their studies compared to what bees will likely encounter when foraging nectars from seed treated crops such as oilseed rape, *Phacelia*, sunflower or alfalfa. It is highlighted that for these calculations the maximum residue values were used and it may be reasonable to assume that honeybees in field realistic situations will likely meet lower nectar concentrations. However, the available residue dataset was limited and the extrapolation from a crop to another could not be performed. Therefore, these results cannot be considered fully representative for all the uses authorised in EU.

3.5.2. Honeybees, comparison of the tested doses with the estimated residue intake

The effective dose of a forager bee depends on several factors and not only on the concentration of a certain bee relevant matrix (nectar in this case). The doses used in the papers were compared with the estimated residue intake assuming the same consumption model as used by Henry *et al*. (2012) from Rortais *et al*. (2005).

It was assumed that the effective amount of a contaminant (that is absorbed by the bee) is directly proportional with the consumed nectar. It has to be highlighted that nectar foragers, although they can collect a large quantity of nectar on a single day or within a few hours, digest only a certain part of it and the rest is brought back to the hive for storage. Their consumption depends on their energy demand, which depends on their activity.

The volume of nectar ingested by foragers per time unit depends on two parameters: 1) the energy needed (i.e. the amount of sugar) for flying per time unit. In Balderrama *et al.* (1992) it was reported to be 8-12 mg of sugar per hour, which equals to 13-19.5 µL, considering 50% sugar content and a density of 1.23 kg/L. Based on the number of trips a forager can make in a day (n=10) and the flight duration of a trip (between 30 and 80 minutes), Rortais *et al.* (2005) estimated that nectar foragers will spend between 4 and 10.7 hour per day for flight activities, and therefore will require 32-128 mg of sugar per day; 2) the sugar content of the nectar on which bees forage. It is depending on the type of crop. It was estimated that the sugar content in nectar may vary between 5-80%, depending on the type of crop considered (Crane, 1975). For example, in sunflower and winter oilseed rape, it is estimated to be on average 40% and 20%, respectively (Pham-Delègue and Bonjean, 1983; Pierre *et al*, 1999). In addition, sugar content in nectar varies in time and space according to several environmental factors.

To estimate the potential amount of active substance contained in the nectar that foragers may collect and ingest per time unit, several scenarios were considered in relation to the above sugar content and the energy demand parameters (**Table 9**).

It is important to note that forager bees may uptake uncontaminated feed items from the hive before leaving to forage. This can lead to some dilution of the contaminants in the stomach content of the bees. Therefore, the residue absorbed by the bee can be lower than those estimated in these scenarios, which assumed that bees consume exclusively contaminated nectar.

Table 9: Potential intake of thiamethoxam, clothianidin and imidacloprid for a nectar forager bee based on the highest nectar residues.

sugar content of nectar %	**thiamethoxam**		**clothianidin**		**imidacloprid**	
	ng/bee		ng/bee		ng/bee	
	per hour flying	To forage a day *	per hour flying	To forage a day *	per hour flying	To forage a day *
10	0.368-0.552	1.472-5.888	0.688-1.032	2.752-11.008	0.065-0.097	0.259-1.037
20	0.184-0.276	0.736-2.944	0.344-0.516	1.376-5.504	0.032-0.049	0.130-0.518
30	0.123-0.184	0.491-1.963	0.229-0.344	0.917-3.669	0.022-0.032	0.086-0.346
40	0.092-0.138	0.368-1.472	0.172-0.258	0.688-2.752	0.016-0.024	0.065-0.259
50	0.074-0.110	0.294-1.178	0.138-0.206	0.550-2.202	0.013-0.019	0.052-0.207
60	0.061-0.092	0.245-0.981	0.115-0.172	0.459-1.835	0.011-0.016	0.043-0.173
70	0.053-0.079	0.210-0.841	0.098-0.147	0.393-1.573	0.009-0.014	0.037-0.148
80	0.046-0.069	0.184-0.736	0.086-0.129	0.344-1.376	0.008-0.012	0.032-0.130

* based on the scenario of 10 trips/day lasting about 30-80 minutes each (Rortais *et al*., 2005)

Based on these calculations, for thiamethoxam the estimated residue intakes by foragers for one hour flying are lower than the dose tested by Henry *et al*. The highest values were: 0.368-0.552 ng thiamethoxam/bee *vs* 1.34 ng thiamethoxam/bee. The daily estimated residue intakes were higher for the scenarios with a sugar content from 10 to 40%, assuming the higher sugar demand (128 mg per day), but was higher only for the scenario with a sugar content of 10% assuming the lower sugar demand (32 mg per day). The highest values calculated were: 1.472-5.888 ng thiamethoxam/bee *vs* 1.34 ng thiamethoxam/bee.

For clothianidin, the estimated residue intakes for one hour flying are higher than the dose tested by Schneider *et al*. in the scenarios with a sugar content from 10 to 20%, assuming the higher sugar demand (12 mg per hour), but was higher only for the scenario with a sugar content of 10% assuming the lower sugar demand (8 mg per hour). The highest values were: 0.688-1.032 ng clothianidin/bee *vs* 0.5 ng/bee. The daily estimated residue intakes were higher for all the scenarios, assuming the higher sugar demand (128 mg per day), but was higher for the scenarios with a sugar content from 10% to 50% assuming the lower sugar demand (32 mg per day). The highest values calculated were: 2.752-11.008 ng clothianidin/bee *vs* 0.5 ng clothianidin/bee.

In the case of imidacloprid the estimated residue intakes based on these calculations are lower than the doses used in the tested by Schneider *et al*. The highest values were 0.259-1.037 ng imidacloprid/bee *vs* 1.5 ng imidacloprid/bee.

It has to be noted however that neither the energy expenditure nor the kinetics of the adsorption of the toxicants in the studies is reliably known. In the studies the bees were starved and confined, their activity cannot be compared with a high energy demand activity like flying. Therefore, the absolute comparison of the doses used by the authors with either of the scenarios considered above might be misleading and should only be considered to be an illustration. The more realistic scenario for the oral exposure of forager bees consist of several small exposure pulses over time or a continuous, but low level exposure.

Overall, it might be concluded that forager bees will not likely be exposed within a few hours to the same doses as those used in the studies, with the exception of the scenarios for clothianidin when the sugar content is from 10% to 20%. However, they can be exposed over longer durations when foraging on contaminated crops for several days or weeks. The estimation of which exposure regime represents higher likelihood of exposure (the rather acute that was used in the studies or a chronic, which is more field realistic) would require some considerations of the toxicodynamics/toxicokinetics of the active substance in bees.

3.5.3. Bumblebees, comparison of the tested concentrations with residue data

As regards the research study on bumblebees from Whitehorn *et al*., the concentrations of the feed items used in the lower treatment levels were slightly below the highest concentrations of imidacloprid found in nectar and pollen (**Table 10**).

Table 10: Comparison of doses used in the studies by Whitehorn *et al*. with the highest residue levels

	Concentration used in the test	**Highest concentration in pollen and nectar**[(a)]
imidacloprid (Whitehorn *et al*.)	6 µg/kg in pollen[(b)] 12 µg/kg in pollen[(c)] 0.7 µg/kg in sugar water[(b)] 1.4µg/kg in sugar water[(c)]	7.6 µg/kg in pollen 0.81 µg/kg in nectar

(a) values from a study conducted in USA, on canola; (b) low treatment levels; (c) highest treatment levels.

On the basis of this comparison, it might be concluded that bumblebees can be exposed to similar concentrations than those used in this study. On the other hand bumblebees in the research study where exposed for 2 consecutive weeks. This exposure time is questionable because in normal field conditions bumblebees would need to forage for two weeks exclusively on imidacloprid-treated crops in order to be exposed to the same extent. However, it could be possible in intensive agricultural landscapes like monoculture areas.

It has to be noted that the maximum residue values used for these comparisons originate from a study conducted on oilseed rape (canola) in USA. Most of the residue data originating from European studies indicated much lower levels even when the application rates in the residue trials were higher than the application rates authorised in EU Member States. The highest values (i.e. in pollen 0.002 mg/kg, in nectar <0.01mg/kg) from field European studies were detected in oilseed rape. In maize or in sunflower no residues in pollen or nectar were detected in field studies. On the other hand it is noted that the majority of the available analytical measurements were not sensitive enough (relatively high quantification and detection limits). Therefore the uncertainty of the representativeness of the residue data for imidacloprid is higher than for other residue values. Overall, on the basis of the available data, considering the uncertainties and the limited dataset, the exposure to higher residues cannot be excluded. It is also noted that it is likely that bumblebees usually will forage for more mixed diets, especially in more complex landscapes.

3.6. Consideration of the possible applicability of the published results to other neonicotinoids

Toxicity endpoints are reported in **Table 11**. Data were derived from studies on *Apis mellifera*, except the endpoint for thiamethoxam formulated product which is from a study on *Bombus terrestris*.

Generally, the oral toxicity appears to be higher than the contact toxicity (one order of magnitude). Imidacloprid, thiamethoxam and clothianidin show a similar acute toxicological profile, while thiacloprid and acetamiprid are less toxic.

Table 11: Acute toxicity endpoints for *Apis mellifera* (source of data: list of endpoint in review reports and DARs).

Active substance	**Acute oral toxicity LD_{50} µg/bee (NOEL)**		**Acute contact toxicity LD_{50} µg/bee (NOEL)**	
imidacloprid	0.0037 (<0.0015)	active substance	0.081 (<0.0025)	active substance
	0.0056	formulated product	0.042	formulated product
thiamethoxam	0.005 (0.002)	active substance	0.024 (0.01)	active substance
	0.02[(a)]	formulated product	0.11[(a)]	formulated product
	0.0168	metabolite CGA 322704	0.0275	metabolite CGA 322704
clothianidin	0.00379 (0.001024)	active substance	0.04426 (0.008)	active substance
	3.9 (0.9)	metabolite TZNG	-	-
thiacloprid	17.32	active substance	38.82	active substance
	8.51	formulated product	51.6	formulated product
acetamiprid	14.53	active substance	8.09	active substance
	8.85	formulated product	9.26	formulated product

(a): *Bombus terrestris*; form.: formulated product.

Thiacloprid and acetamiprid are cyano-substituted neonicotinoids while clothianidin, imidacloprid and thiamethoxam are nitroguanidine-substituted neonicotinoids. There are data to suggest that the former are readily metabolised in bees and they have considerably lower acute toxicity profiles for bees than the nitroguanidine-substituted neonicotinoids.

Considering the toxicity of these substances, the sub-lethal effects observed on honeybees and bumblebees for imidacloprid, thiamethoxam and clothianidin are not likely to occur for thiacloprid and acetamiprid at similar levels of exposure as those tested by Henry *et al*., Schneider *et al.* and Whitehorn *et al*.

CONCLUSIONS

On the basis of the data considered in this statement, the comparison between the doses tested in the studies with the actual doses to which bees may be exposed, based on the supported uses at EU level and the authorisations granted by Members States, was only possible for the seed treatment uses to maize, sunflower, oilseed rape and alfalfa.

As regards to honeybees:

> On the basis of the comparison between the concentrations tested in the published studies and the highest residue levels, it can be concluded that tested concentrations were higher than the concentrations of thiamethoxam, clothianidin and imidacloprid found in nectar of the above mentioned seed treated crops and *Phacelia*.
>
> On the basis of the comparison between the doses used in the studies and the estimated hourly residue intake by consumption of contaminated nectar, it can be concluded that bees will likely not be exposed to higher doses than those used in the studies, with the exception of some scenarios for clothianidin. The estimated daily intake indicated that, for thiamethoxam and clothianidin the exposure can be higher than the tested doses. However, it is important to note that neither the energy expenditure nor the kinetics of the adsorption of the toxicants in the studies is reliably known. Therefore, the scenarios for estimating the residue intake presented in the current document should be viewed with caution but have been provided for illustrative purposes.

As regards to bumblebees:

> The concentrations tested were in the range of the maximum residues of imidacloprid measured in pollen and nectar. However, it is uncertain as to what extent exposure situation in the study is representative of field conditions, since bumblebees would need to forage for two weeks exclusively on imidacloprid-treated crops in order to be exposed to the same extent as in the study. Further consideration would be necessary to understand whether this situation may occur in intensive monoculture landscapes.

As regards to the applicability of the new results to other neonicotinoids used as a seed treatment:

> It is noted that Member States did not report any authorised uses for acetamiprid as a seed treatment. Furthermore, due to the differing acute toxicity observed in standard laboratory studies, the sub-lethal effects observed for honeybees and bumblebees for imidacloprid, thiamethoxam, clothianidin are not considered to likely occur for thiacloprid and acetamiprid at similar levels of exposure as those tested.

Overall, before drawing definite conclusions on the behavioural effects regarding sub-lethal exposure of foragers exposed to actual doses of neonicotinoids and the consequences to the colony it would be necessary to repeat the experiments performed in the studies with other exposure levels or in other situations. In addition, further data would be necessary to fully consider the relevance of the results of the new research studies to seed treatment of other crops and to spray uses. Also considerations of the toxicodynamics/toxicokinetics of thiamethoxam and clothianidin in bees would be necessary to fully validate the residue intake estimations.

EFSA recently received a mandate from the European Commission for scientific and technical assistance and was requested to provide an EFSA conclusion with an updated risk assessment to bees for these active substances: thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid. Particular attention will be given to acute and chronic effects of colony survival and development, taking into account effects on bee larvae, bee behaviour. In this context, an assessment of effects of sub-lethal doses on bee survival and behaviour will be further considered.

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ABBREVIATIONS

µg	microgram
µL	microlitre
a.s.	active substance
d	day
DAR	Draft Assessment Report
EU	European Union
g	gram
GAP	good agricultural practice
h	hour(s)
ha	hectare
kg	kilogram
L	litre
LD_{50}	lethal dose, median; dosis letalis media
LOD	limit of detection
LOQ	limit of quantification
mg	milligram
mL	millilitre
MS	Member State
ng	nanogram
NOEL	no observed effect level
ppb	parts per billion
RFID	radiofrequency identification
wk	week
yr	year