STATEMENT OF EFSA

Evaluation of the FERA study on bumble bees and consideration of its potential impact on the EFSA conclusions on neonicotinoids[1]

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ABSTRACT

The European Food Safety Authority was requested to clarify whether the new publication on the effects of neonicotinoid seed treatments on bumble bee colonies under field conditions (March, 2013; Thompson *et al.*) has an impact on the EFSA Conclusions on the three neonicotinoids clothianidin, thiamethoxam and imidacloprid (EFSA Journal 2013;11(1):3066; EFSA Journal 2013;11(1):3067; EFSA Journal 2013;11(1):3068). The Conclusions on neonicotinoids, published on 16 January 2013, did not permit to perform a risk assessment for bumble bees and identified the need for further information to address the risk to pollinators other than honey bees. The conclusions of this scientific statement were reached on the basis of the evaluation of the study report by Thompson *et al.* (2013), and additional raw data made available by the study authors to EFSA. The study investigated the exposure of bumble bee colonies placed in the vicinity of crops treated with neonicotinoids and its major effects on bumble bee colonies. The current assessment concluded that, due to the weaknesses of the study design and methodology, the study did not allow to draw any conclusion on the effects of neonicotinoids on exposed bumble bee colonies, and confirmed that the outcome of the conclusions drawn for the three neonicotinoid insecticides remains unchanged.

KEY WORDS

Neonicotinoids, bumble bees, field study, oilseed rape, risk assessment

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SUMMARY

In March 2013 a new study was published by the UK Food and Environment Research Agency (FERA) investigating the effects of neonicotinoid seed treatments on bumble bee (*Bombus terrestris*) colonies under field conditions (March, 2013; Thompson *et al.*). The study investigated effects on bumble bee colonies placed in the vicinity of crops treated with neonicotinoids. The authors concluded that the study did not show conclusively that exposure to neonicotinoids, used within a normal agricultural setting, had a major effect on bumble bees colonies.

The European Food Safety Authority (EFSA) was requested by the European Commission to clarify whether this new study had an impact on the risk assessment for bees provided in the EFSA Conclusions on the three neonicotinoids clothianidin, thiamethoxam and imidacloprid (EFSA Journal 2013;11(1):3066; EFSA Journal 2013;11(1):3067; EFSA Journal 2013;11(1):3068).

To address the request from the European Commission, EFSA performed an evaluation of the study by Thompson *et al.* (2013) by taking into account the study report and the additional raw data submitted by the study authors upon request from EFSA. EFSA performed an in-depth assessment of the study, particularly focusing on the statistical methodology used.

Furthermore, the routes and level of exposure in Thompson *et al.* (2013) in relation to those assessed in the EFSA Conclusions on the three neonicotinoids clothianidin, thiamethoxam and imidacloprid were considered. Finally, the suitability of field studies performed with bumble bees for understanding the risk to honey bees and solitary bees was discussed.

EFSA identified several weaknesses of the study design and in particular the lack of an unexposed control, and uncontrolled covariates. In addition, EFSA noted that the route and level of exposure in the Thompson *et al.* (2013) study was not adequate to address the risks to honey bees for the authorised uses as indicated in the EFSA Conclusions. EFSA also considered that field studies performed with bumble bees cannot be used to understand the risk for honey bees and solitary bees.

Overall, EFSA considered that the study is not adequate to understand the effects of exposure of neonicotinoid residues on bumble bee colonies. EFSA also concluded that the study by Thompson *et al.* (2013) does not change the conclusions of the risk assessment previously drawn for thiamethoxam, clothianidin and imidacloprid in the EFSA Conclusions published in January 2013 (EFSA 2013a, EFSA 2013b and EFSA 2013c).

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

In March 2013 a new study was published by the UK Food and Environment Research Agency (FERA) investigating the effects of neonicotinoid seed treatments on bumble bee (*Bombus terrestris*) colonies under field conditions (March, 2013; Thompson *et al.*). The study tested the hypothesis that exposure of bumble bee colonies placed in the vicinity of crops treated with neonicotinoids had no major effect on the health of the colonies. For this purpose, the development of bumble bee colonies placed in three sites near oilseed rape crops grown from untreated seeds, or from seeds treated with the neonicotinoid insecticides clothianidin or imidacloprid was investigated.

On the basis of the results, Thompson *et al.* (2013) concluded that "*within this context, the study did not show conclusively that exposure to neonicotinoids used within a normal agricultural setting had major effects on bumble bees colonies*".

On 16 January 2013 EFSA published the EFSA Conclusions on the revised risk assessments for bees for the three neonicotinoids, thiamethoxam, clothianidin and imidacloprid (EFSA, 2013a, 2013b and 2013c). The risk assessments considered exposure to dust (generated during the sowing of seed), contaminated nectar and pollen, and guttation fluid for the authorised uses as seed treatment and granules in the EU. A high risk was indicated, or could not be excluded, for certain aspects of the honey bee risk assessment for a number of the authorised uses and several data gaps were identified. Furthermore, the risk assessment for pollinators other than honey bees could not be finalised.

The aim of this scientific statement is to investigate the relevance of the Thompson *et al.* (2013) study and its impact on the three recently published EFSA Conclusions on neonicotinoids.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

On 27 March 2013 EFSA received a request from the European Commission for scientific and technical assistance concerning a new study on neonicotinoid seed treatments made available by the UK Food and Environment Research Agency (FERA): "Effects of neonicotinoid seed treatments on bumble bee colonies under field conditions" (March, 2013; Thompson *et al.*).

In particular, EFSA was requested by the European Commission to provide a scientific statement clarifying whether the new publication has an impact on the EFSA Conclusions on the three neonicotinoids thiamethoxam, clothianidin and imidacloprid, which were published on 16 January 2013 (EFSA 2013a, EFSA 2013b, EFSA 2013c).

The agreed deadline for providing the statement is 31 May 2013.

CONTEXT OF THE SCIENTIFIC OUTPUT

The context of the evaluation and production of this scientific output was that required by the European Commission in accordance with Article 21 of Regulation (EC) No 1107/2009 concerning the review of approval of active substances in the light of new scientific and technical knowledge or monitoring data.

To address the request from the European Commission, in this scientific statement EFSA performed an evaluation of the FERA study by taking into account the study report and the additional raw data submitted by the study authors upon request from EFSA. Subsequently, the relevance of this study regarding the conclusions drawn for the three neonicotinoid insecticides as published on 16 January 2013 was considered.

Furthermore, in this context an evaluation of the statistical analysis and methodology used in the interpretation of the results was undertaken by the EFSA Scientific Assessment Support (SAS) Unit and presented in the Appendix to this statement.

EVALUATION

1. Thompson *et al.* (2013) study overview

Bumble bee (*Bombus terrestris* Audax) colonies were placed adjacent to flowering oilseed rape fields at three separate locations in northern England, namely site A, site B and site C. The oilseed rape at site A was stated to have not been treated with any neonicotinoid active substance, while the oilseed rape at sites B and C had been grown from seeds treated with a plant protection product containing the neonicotinoid active substance clothianidin or imidacloprid, respectively.

After the flowering period of the oilseed rape in the test fields the bumble bee colonies were moved to sites where the surrounding plants had not been treated with neonicotinoids. It was stated that the flowering period of the oilseed rape was longer than anticipated, which was speculated to be due to cool temperatures. Weekly assessments were made on colony mass and foraging activity. Temperature was recorded at each study site.

The colonies were maintained for a total period of 8 - 9 weeks and were allowed to reach the same developmental stage. At that time point the colonies were freeze-killed and then dissected for further assessment. The number and mass of queens (gynes), drones, workers, larvae, pupae, and the number of eggs, nectar and pollen storage cells present were recorded. Due to the later placement of the colonies at site C, the colonies at site C were killed two weeks later than at sites A and B. The presence of spores of *Nosema bombi* and *Crithidia bombi* in the queens was assessed by microscopy.

To analyse the residue levels of the active substances under investigation and their toxic metabolites in the study areas, samples of nectar and pollen were collected from the bumble bee colonies and from the flowering crop. The sampling from bumble bee colonies was performed during the peak flowering period of the oilseed rape. Sampling of nectar and pollen from the flowering crops was carried out by taking samples from the comb of a small honey bee colony, which was placed in the field confined in a mesh tent. To investigate the pollen origin (palynological analysis), pollen was collected from returning bumble bee foragers.

Statistical analyses were performed on a number of the biological parameters.

Thompson *et al.* (2013) acknowledged weaknesses in the study design and methodology. In particular, Thompson *et al.* (2013) highlighted that the lack of replication, the variability between test sites and the presence of other neonicotinoids at the proposed control site as well as the two test sites, meant that a formal statistical test of the (null) hypothesis was not possible. Thompson *et al.* (2013) proposed that the results of the study are reassuring but should not be regarded as definitive. Nevertheless, the authors did indicate a number of conclusions:

1. Thompson *et al.* (2013) indicated that they have shown that bumble bee colonies remained viable and productive in the presence of neonicotinoid residues under the conditions of the study.

2. Thompson *et al.* (2013) suggested that the results indicate no consistent relationship between neonicotinoid residues in pollen and nectar and an effect on colony mass at the time when the residue sample was taken, or at study termination, or on the number of queens produced.

3. Thompson *et al.* (2013) proposed that they would have expected to identify a clear relationship if exposure to neonicotinoids was a ‘major source of field mortality and morbidity’ of bumble bee colonies.

4. Thompson *et al.* (2013) proposed that their study highlights the importance of taking care in extrapolating laboratory based experiments to field conditions.

2. In-depth review of the Thompson *et al.* (2013) study

2.1. Study objective

EFSA noted several inconsistencies and contradictory statements regarding the study objectives.

It was indicated that the intention was to address the concerns raised by the results of the Whitehorn *et al.* (2012) study by 'extending it to the field'. The research performed by Whitehorn *et al.* (2012) focused on the effects of sublethal doses of imidacloprid on bumble bee colonies. However, the study design of the Thompson *et al.* (2013) study did not replicate the design of the experiment by Whitehorn *et al.* (2012) under field conditions. The study design used by Thompson *et al.* (2013) introduced a number of additional variables, including exposure to other neonicotinoids, which were not accounted for in Whitehorn *et al.* (2012). As the colonies were placed in a general agricultural landscape, it was not possible to control several variables, which may affect the development of the bumble bee colonies (exposure to other pesticides, variable sources of nectar and pollen).

It was also indicated that the aim was to test the hypothesis that "*exposure of bumble bee colonies placed in the vicinity of crops treated with neonicotinoids had no major effects on the health of the colonies*". However, it is not clear whether the study aimed to test a specific hypothesis, or whether the study was just to investigate a number of bumble bee parameters under field conditions (*i.e.* a more standard ecology field study). The term 'major effect' should have been clearly defined and the assessments that would be performed to identify such an effect should have been described (EFSA, 2011). For example, it was not clear if 'major effect' related to effects on queen production, foraging behaviour or colony mortality/morbidity. Further consideration of the defined study objectives in relation to the statistical methodology is reported in the Appendix of this statement.

The active substances under investigation were imidacloprid and clothianidin. However, following unintentional contamination of the test site A (proposed control site) by another neonicotinoid, thiamethoxam, as a secondary objective (identified while the study was ongoing), an analysis was performed to attempt assessing the effects of exposure of thiamethoxam to bumble bee colony parameters (number of queens and colony mass).

2.2. Study methodology

Several aspects of the materials and methods were lacking in detail, such as the amount of active substance in the products, details of the application of the plant protection products to the seeds, details of the drilling of the seeds at the test sites, details of other non-neonicotinoid plant protection products applied to the test fields and the surrounding fields, more detailed reporting of the crop growth-stage assessments, more precise reporting of the surrounding area surveys, and further details of post-exposure location of the bumble bee colonies.

The climatic conditions at each test site were poorly reported or missing, e.g. rainfall at each test site. It was also noted that a number of the recorded temperatures seem to be abnormally high for the UK (maximum of 38.7°C recorded at site C on the 26 June 2012).

The protocol for collection of pollen and nectar samples was neither fully explained nor included in the study report.

The results of some assessments, which were stated to have been performed, were not fully reported or were missing in the study report. Specifically, it would have been expected that the following would have been reported: details of the weekly transect foraging assessments, results of the disease analysis, raw data for residue analysis from honey bee colonies, condition of the honey bee combs prior to exposure (specifically the quantity of stored nectar and pollen), and a method to determine newly stored pollen and nectar.

2.3. Field sites and environmental conditions

Twenty queen-right bumble bee (*Bombus terrestris* Audax) colonies were randomly assigned (except at site C) and placed adjacent to flowering oilseed rape fields at three separate locations in northern England. Details of the field sites and pesticide treatments are given in Table 1.

Table 1: Details of study sites and pesticide treatments applied in Thompson *et al.* (2013)

	Site A	Site B	Site C
Plant protection product applied to the seed	**Proposed control**[2] Seed not treated with a neonicotinoid active substance	**'Modesto'** (containing 80 g/L beta-cyfluthrin and 400 g/L clothianidin)[1]	**'Chinook'** (containing 100 g/L beta-cyfluthrin and 100 g/L imidacloprid)[1]
Seed treatment rate of neonicotinoid active substance (g a.s./kg seed)	-	5 g clothianidin/kg seed	2 g imidacloprid/kg seed
Seed sowing rate	3.5 kg seeds/ha	3 kg seed/ha	5.41 kg seed/ha
Application rate of neonicotinoid active substance (g a.s./ha)	-	15 g clothianidin/ha	11 g imidacloprid/ha
Seed variety	Catana (conventional)	Excalibur (hybrid)	Catana (conventional)
Size of field	6.5 ha	10.7 ha	12.1 ha
Date of exposure	13 April – 2 June	13 April – 2 June	26 April – 11 June[3]

[1] UK Chemical Regulation Directorate (CRD) Pesticides Register Database (accessed on 26/4/13). Information was not clearly reported in Thompson *et al.* (2013)
[2] For reasons discussed in section 2.4, site A should not be referred to as a control
[3] Colonies at site C were placed adjacent to the treated field 13 days later than in case of sites A and B due to later flowering of the crop at location site C

The number of bumble bee colonies, which were placed at each test site (twenty per site), is considered reasonable. However, only one study site was used for the proposed control, the 'Modesto' treated seed and the 'Chinook' treated seed. A lack of site replication is considered to be a weakness of the study design, which was acknowledged by Thompson *et al.* (2013), and is further discussed in relation to the statistical analyses in the Appendix of this statement.

It is noted that different seed varieties were used at the study sites (see Table 1). As acknowledged by Thompson *et al.* (2013), seed variety can influence the attractiveness of oilseed rape to honey bees (*Apis mellifera*) for foraging. It would be reasonable to speculate that this may also be true for bumble bees. No information was reported in the study to address the impact of this potential variable.

General information was reported regarding the crops and other flowering plants growing in the surrounding area at each study site (including the presence of oilseed rape). Some information was included on the presence of neonicotinoid active substances (thiamethoxam, clothianidin and imidacloprid) in the vicinity of each study site, however, details were only reported for the test fields (e.g. application rate). For test sites B and C, crops grown from neonicotinoid treated seeds were present in the surrounding area. In addition, no information on the use of other pesticides applied at the study sites or in the surrounding area was reported. It is noted that all the above mentioned information, including the information on the plant protection products for clothianidin and imidacloprid, was gathered from farmers and agronomists rather than being controlled by the study authors, which is considered to be a source of uncertainty for understanding exposure of the bumble bees.

It is considered that the test sites and surrounding areas reflect a small sample of agricultural conditions in the UK. However, no assessment of whether the surrounding crops are representative of normal crop situations in the UK or other Member States was included. Therefore, it is considered that the test sites cannot be deemed to be fully representative for other EU agricultural conditions.

2.4. Residue sampling and analysis and palynological assessments

Pollen and nectar from bumble bee colonies were sampled on a single day, either 25 or 26 days after the colonies were placed in the fields. This may not be sufficient to characterise the exposure to imidacloprid and clothianidin during the study duration. For example, a more representative estimate of exposure from the treated field may have been obtained if the pollen was collected at the beginning of the flowering period, when samples were less likely to be contaminated from other fields. In addition, it would have been expected that the historical use of neonicotinoids in the treated field would have been reported.

As regards the pollen and nectar collected from honey bee combs, the study authors reported that the honey bees escaped from the mesh tents. The tents were intended to limit honey bee foraging in the treated crop and therefore the honey bee samples are not representative for the study sites. This was confirmed by the results of the honey bee residue analyses, where additional neonicotinoids were detected (*i.e.* different to the neonicotinoid used on the treated field) (see below). In addition, since the condition of honey bee combs prior to exposure (specifically the quantity of stored nectar and pollen) was not reported, the residue data from honey bee samples are considered to be of limited value, as also acknowledged by the study authors.

The palynological analysis indicated that bumble bees forage on different crops, and that oilseed rape pollen contributed 13 - 26 % in terms of mean percentage of pollen mass returned to the colonies. By excluding the samples where oilseed rape pollen was not detected, the mean contribution was 35 – 37 %. These results may be considered as indicating that under "real field conditions" bumble bees used a variety of sources of pollen and nectar, which could limit their potential exposure to contaminated oilseed rape nectar and pollen. Whilst this finding is useful to conclude on the potential exposure within a small sample of field conditions (similar to those of the study sites), it cannot be considered sufficient to understand the extent of the exposure to neonicotinoids and hence effects on bumble bee colonies in other situations (e.g. monoculture landscapes).

The residues of clothianidin, thiamethoxam and imidacloprid (and its toxic metabolites) in nectar and pollen samples were determined by liquid chromatography-mass spectrometry. The authors stated that the method was not fully validated for thiamethoxam. However, EFSA noted that, from the limited validation data available, it was not possible to conclude on the acceptability of the method for the determination of thiamethoxam. The authors indicated that the LOD in nectar was 0.025 – 0.05 µg/kg and in pollen it was 0.5 µg/kg (for all three test substances). The LOQ for imidacloprid and clothianidin in nectar was 0.16 µg/kg and in pollen it was 0.5 µg/kg. However, EFSA noted that the LOQ for thiamethoxam was not adequately supported and therefore there is uncertainty related to the results of the residue analysis for thiamethoxam.

The results of the residue analysis are summarised as follows:

- Site A (proposed control): thiamethoxam was detected above the LOQ in nectar and pollen collected from bumble bee colonies; clothianidin was above the LOD (but less than the LOQ) in nectar but not in pollen. Imidacloprid was not detected above the LOD in nectar or pollen. Residues of thiamethoxam above the LOQ were also detected in pollen from honey bee comb.

- Site B: thiamethoxam was detected above the LOQ in nectar and pollen collected from bumble bee colonies. Clothianidin was detected above the LOD (but less than the LOQ) in nectar but not in pollen. Imidacloprid was not detected above the LOD in nectar or pollen in bumble bee samples. Residues of thiamethoxam above the LOQ in pollen, clothianidin above the LOQ (nectar and pollen) and imidacloprid (nectar) were also detected in samples from honey bee comb.

- Site C: thiamethoxam was not detected; residues of clothianidin and imidacloprid were above the LOD (but less than the LOQ) in nectar from bumble bee colonies, and clothianidin was above the LOQ in nectar from honey bee comb.

Bumble bee foraging distances range from a few metres from the colony up to 1.5 km (Osborne *et al.* 1999, Walther-Hellwig and Frankl, 2000, Thompson *et al.* 1999, Osborne *et al.* 2008). Therefore, exposure to pesticides applied to crops beyond the test fields is expected. The results of the residue analysis of the pollen and nectar taken from the bumble bees and bumble bee colonies confirmed that the bumble bees were exposed to a mixture of neonicotinoid pesticides.

The exposure of the bumble bees to neonicotinoid pesticides at test site A (proposed as the control) severely limited the reliability of the study (see also Appendix). Furthermore, given that the bees at test sites B and C were exposed to a mixture of neonicotinoid pesticides, it would not be possible to differentiate the cause of an observed effect. Thompson *et al.* (2013) indicated that the study was established in a very short time-scale, which may account for the difficulties in finding suitable test sites. Nevertheless, it would have been preferable if the test site selection ensured that there were no other fields treated with neonicotinoids in the vicinity.

2.5. Bumble bee observations

The bumble bee colonies used in the study were obtained from a commercial supplier. At the start of the exposure period, when the colonies were placed at the test sites, the mean size and weight of the colonies were as follows:

Site A: contained 21 ± 2 worker bees and weighed 0.579 ± 0.003 kg;

Site B: contained 24 ± 2 worker bees and weighed 0.578 ± 0.003 kg;

Site C: contained 16 ± 1 worker bees and weighed 0.546 ± 0.002 kg.

As noted by Thompson *et al.* (2013), the initial number of worker bees in the colonies at site C was significantly different ($p=0.04$) compared to the colonies placed at sites A and B. Although this was stated to have been accounted for in the statistical analyses (see Appendix), it would have been preferable to ensure that the initial colony size was more uniform across the three test sites.

Thompson *et al.* (2013) stated that low foraging activity was observed in the test fields. The low level of foraging activity indicates that the potential exposure of the bumble bees to the test items (clothianidin and imidacloprid) was relatively low. These results are in line with the palynological analysis, which indicated a low proportion of oilseed rape pollen collected by the bumble bees (see section 2.4). The results of the assessment of bumble bee movement in and out of the colonies were presented graphically in the study report (figure 4, page 16 of Thompson *et al.*, 2013), but the raw data were not included. Following a request, Thompson *et al.* (2013) provided this information to EFSA and the results confirm that the bumble bees were active at each colony.

A number of differences in the parameters investigated were observed between the colonies at site C and the colonies at sites A and B. The colony mass gain at site C was statistically significantly lower than that at sites A and B, and a similar trend was observed for a number of colony structure parameters by accounting for the "adjusted" values. For example, a lower percentage of queen number, queen pupae, larvae occupancy, drone and worker pupae and number of eggs was observed. The authors suggest that these differences are likely to be due to the conditions at site C limiting the development of the colonies. However, without a control and replicates, EFSA considers that it is not possible to reach a conclusion regarding the reasons for the observed differences in the colony development at site C. It is noted that residues of imidacloprid were detected between the LOD and the LOQ in nectar collected from the bumble bee colonies at site C, but they were not detected in bumble bees at test sites A and B.

To address the secondary objective, Thompson *et al.* (2013) performed an analysis to assess the effects of exposure to thiamethoxam and its metabolite clothianidin (separately) on bumble bee colony parameters (number of queens and colony mass). The authors concluded that no consistent relationship was identified between residues of thiamethoxam in pollen and nectar, and queen production and

colony mass gain. The authors stated that residues of thiamethoxam were a common variable between the three test sites, however, considering the residue results provided in Table 6 of the study report, it is noted that thiamethoxam was not detected at test site C. Given that the colonies at all three test sites were exposed to a mixture of neonicotinoids and there was no unexposed control, EFSA does not consider that a correlation between residue of a single neonicotinoid and effect on the colony is meaningful. Furthermore, as discussed in the Appendix of this statement, EFSA also raised a number of specific concerns regarding the statistical approach taken in the residue-based analysis (e.g. removal of data as outliers).

2.6. Overall EFSA conclusion on the Thompson *et al.* (2013) study

On the basis of the results, Thompson et al. (2013) concluded that "*Overall, there were no consistent relationships between neonicotinoid residues in pollen and nectar with colony mass at the time of sampling or at the end of the study or with the numbers of queens produced. Within this context, the study did not show conclusively that exposure to neonicotinoids used within a normal agricultural setting had major effects on bumble bees colonies".*

EFSA considers that:

- The objectives (problem formulation) were not clearly defined therefore it is difficult to determine whether the study design was fit for the purpose of the study.
- The lack of detailed reporting of the materials and methods creates uncertainty in the interpretation of the findings of the study.
- Suitable control colonies were not available.
- The variability of the initial colony size and a lack of uniformity of influential parameters important for colony development reduces the reliability of the study to detect differences in colony development between the three test sites.
- Some uncertainties were noted with the sampling and methodology for the pollen and nectar residue analyses.
- Concerns were raised regarding the elaboration and interpretation of the results to reach the proposed conclusions of Thompson *et al.* (2013).

Due to the above weaknesses identified with the study design and methodology, overall EFSA considers that the study is not suitable to draw any conclusion on the relationship between exposure to neonicotinoids, used within a normal agricultural setting, and effects on bumble bees colonies.

3. Impact on the risk assessment performed in the EFSA Conclusions for thiamethoxam, clothianidin and imidacloprid (EFSA, 2013a, 2013b, 2013c)

3.1. Exposure in the Thompson *et al.* (2013) study *vs* exposure from the uses of neonicotinoids

The Conclusions on thiamethoxam, clothianidin and imidacloprid reconsidered the risk assessment for bees (*i.e.* the acute risk and the long-term risk to colony survival and development, including the risk to bee brood, and the risk following exposure to sublethal doses) for the EU authorised uses as seed treatment and granules. The routes of exposure, which were primarily considered, were dust (during the sowing of the treated seed and application of granules), consumption of contaminated nectar and pollen, and guttation fluid.

The authorised uses of thiamethoxam, clothianidin and imidacloprid, considered in the EFSA Conclusions, included several crops and a number of plant protection products as reported in the Appendix A of EFSA 2013a, EFSA 2013b and EFSA 2013c. The study by Thompson *et al.* (2013)

considered only winter sown oilseed rape and two plant protection products (one containing clothianidin and one containing imidacloprid), which are authorised in the UK (Table 1). Clothianidin and imidacloprid have been authorised for oilseed rape seed treatment in several plant protection products in the EU (EFSA 2013b, EFSA 2013c). In terms of the application rates per hectare (*i.e.* a combination of seed sowing rates and seed dressing rates), the range of the maximum application rates authorised for clothianidin was 25 - 80 g a.s./ha and for imidacloprid 10 - 52.5 g a.s./ha. According to the information reported by Thompson *et al*. (2013), the application rate for clothianidin was 15 g a.s./ha and for imidacloprid 11 g a.s./ha. Therefore the application rates used in Thompson *et al*. (2013) do not fully cover the authorised GAPs considered in the EFSA Conclusions. Furthermore, it is important to highlight that the information on the plant protection products for clothianidin and imidacloprid was gathered from farmers and not controlled by the authors of the study (as discussed in section 2.3). Therefore, this should be considered as a source of uncertainty. No sufficient information was reported on the use of thiamethoxam and therefore it is not possible to establish a link between the measured residues and an authorised GAP.

The maximum residue levels in nectar and pollen estimated for the authorised uses of thiamethoxam, clothianidin and imidacloprid in EFSA 2013a, EFSA 2013b and EFSA 2013c can be compared with the maximum residues measured by Thompson *et al.* (2013) (Table 2). It is noted that, in general, the residue levels measured in the study are lower than those estimated in the EFSA 2013a, EFSA 2013b and EFSA 2013c, with the exception of thiamethoxam in nectar in bumble bee samples, which was in the same range at site A, and higher at site B. Care must be taken in comparing maximum residue values as it may not provide a realistic comparison of exposure since it does not account for the distribution of residues (*i.e.* overall exposure). Moreover, as the residue samples were only taken on a single day in the Thompson *et al.* (2013) study, it is not known whether the reported values are a true reflection of the 'maximum' residues. Without an assessment of the real exposure to bumble bees, the results of the study are of limited use for risk assessment as it is not possible to make a comparison to the predicted exposure of bumble bees over a wider area (*i.e.* comparison to worst case or 90th percentile exposure estimations). Furthermore, it is noted that Thompson *et al.* (2013) did not include an assessment of whether the circumstances are comparable to a "realistic worst case" scenario or whether the exposure was "best case". Nevertheless, EFSA notes that the information available suggests that bumble bees (in some situations in Europe) may be exposed to higher residues than those detected by Thompson *et al.* (2013).

Table 2: Maximum residue levels in nectar and pollen estimated for the authorised uses of thiamethoxam, clothianidin and imidacloprid in the EFSA 2013a, EFSA 2013b and EFSA 2013c, and residues measured by Thompson *et al.* (2013)

		Thiamethoxam		**Clothianidin**		**Imidacloprid**	
		nectar	**pollen**	**nectar**	**pollen**	**nectar**	**pollen**
Residue at application rate (µg/kg)[1,2]	**lowest**[1]	0.65	4.59	5.00	5.95	1.59	1.56
	highest[2]	2.72	19.29	16.00	19.04	8.35	8.19
Site A Maximum residue from bumble bees		**1.534**	1.145	0.108	<0.5	<0.025	<0.5
Site A Mean[3] residue from honey bees		-	2.301	-	<0.5	-	<0.5
Site B Maximum residue from bumble bees		**3.877**	1.55	0.283	<0.5	<0.025	<0.5
Site B Mean[3] residue from honey bees		<0.05	2.723	0.053	0.718	0.450	<0.5
Site C Maximum residue from bumble bees		<0.05	<0.5	0.043	<0.5	0.089	<0.5

	Thiamethoxam		Clothianidin		Imidacloprid	
	nectar	**pollen**	**nectar**	**pollen**	**nectar**	**pollen**
Site C Mean[3] residue from honey bees	<0.05	<0.5	0.131	<0.5	0.133	<0.5

[1] Lowest authorised application rate (g a.s./ha) to oilseed rape (EFSA 2013a, EFSA 2013b and EFSA 2013c)
[2] Highest authorised application rate (g a.s./ha) to oilseed rape (EFSA 2013a, EFSA 2013b and EFSA 2013c)
[3] Only mean values from honey bee samples were provided in Thompson *et al.* (2013)
- No results given in Thompson *et al.* (2013)

The bumble bee colonies were placed in the field at the beginning of the flowering period of the winter oilseed rape, therefore the potential exposure from contaminated dust generated during the sowing was not addressed. For treated seeds drilled in the spring, exposure to dust may coincide with the most vulnerable life stage of the bumble bee colony, *i.e*. when the bumble bee queens emerge and need to find a source of food. Furthermore, as reported in EFSA 2013a, EFSA 2013b and EFSA 2013c, guttation is likely to occur more frequently in the early growth stage of the plants. Therefore, this potential route of exposure cannot be considered addressed by the study of Thompson *et al.* (2013).

3.2. Bumble bees *vs* honey bees and other pollinators

In the EFSA Conclusions on neonicotinoids (EFSA 2013a, EFSA 2013b and EFSA 2013c) a general data gap was identified to further address the risk to pollinators other than honey bees (*i.e.* including bumble bees and solitary bees), due to the lack of data. The study by Thompson *et al.* (2013) focused only on bumbles bees and in particular on *Bombus terrestris*. Numerous species of both bumble bees and solitary bees are found in Europe (Williams, 1998, Corbet *et al.* 1991), and currently it has not been agreed what species should be considered in a risk assessment for plant protection products. In terms of ecology, Thompson *et al.* (1999) reported that bumble bee species can have similar habitats. However, Gathmann *et al.* (2002) reported that the ecology of solitary bees could differ from bumble bees and honey bees in terms of habitat and foraging ranges. Overall, in addition to the weaknesses discussed above, the study by Thompson *et al.* (2013) is not considered sufficient to address the general data gap on "pollinators other than bees" identified in the EFSA Conclusions (EFSA 2013a, EFSA 2013b and EFSA 2013c).

In the EFSA Conclusions on neonicotinoids a detailed risk assessment was performed for honey bees. Honey bees differ from bumble bees in terms of physiological, morphological and behavioural characteristics, which could increase or decrease the risk from pesticides (EFSA PPR, 2012). Bumble bees are opportunistic foragers and are known to forage from a wide variety of plants, which is supported by the palynological assessments performed in Thompson *et al.* (2013) (see section 2.4). Furthermore, in Dramstad and Fry (1995) it is reported that bumble bees have a preference for perennial plants in arable landscapes. Honey bee foragers are understood to communicate information regarding available food sources to other forager bees. This is not the case for bumble bees, where it is thought that they will independently learn where to forage (Thompson *et al.* 1999). This difference in behaviour may be important for the assessment of pesticides as it could be expected that, in comparison with bumble bees, a higher proportion of honey bee foragers from a colony could forage on a single crop.

Limited and contradictory information is available regarding the differences in sensitivity of honey bees and bumble bees to pesticides. For example, Thompson *et al.* (1999) considered that, in general, honey bees are more sensitive to pesticides than bumble bees, based on acute oral and contact toxicity endpoints. Mommaerts *et al.* (2011) reported that honey bees were more sensitive to imidacloprid based on acute toxicity values (it was not specified whether this was based on oral or contact endpoints). Cresswell *et al.* (2012) investigated the relative sensitivity of bumble bees and honey bees to imidacloprid by measuring effects on feeding rate, locomotor activity and longevity. Cresswell *et al.* (2012) observed that bumble bees were more sensitive to effects on feeding rate to dietary imidacloprid than honey bees. It is unclear whether an extrapolation of toxicity endpoints can be performed between honey bees and bumble bees. Furthermore, it should be noted that other life stages may also differ in relative sensitivity.

Overall, it is concluded that bumble bee field studies cannot be used to understand the risk to honey bees and solitary bees (and *vice versa*).

CONCLUSIONS

Due to the weaknesses of the study design, in particular the lack of an unexposed control, and uncontrolled covariates, EFSA considers that the study is not adequate to understand the effects of exposure to neonicotinoid residues on bumble bee colonies.

As regards the impact of the study by Thompson *et al.* (2013) on the risk assessment performed in the EFSA Conclusions on clothianidin, thiamethoxam and imidacloprid (EFSA 2013a, EFSA 2013b and EFSA 2013c), the following points are concluded:

- Field studies performed with bumble bees cannot be used to understand the risk for honey bees and solitary bees due to differences in ecology and pesticide sensitivity;
- Some potential routes of exposure (*i.e.* dust and guttation) were not addressed by the study;
- The potential exposure via consumption of contaminated nectar and pollen was assessed only for winter oilseed rape and for two plant protection products, whilst the EFSA Conclusions considered several other crops and plant protection products;
- The study is not considered adequate to address the data gap identified for 'pollinators other than honey bees'.

Overall, it is concluded that the study by Thompson *et al.* (2013) does not change the conclusions of the risk assessment previously drawn for thiamethoxam, clothianidin and imidacloprid in the EFSA Conclusions published in January 2013 (EFSA 2013a, EFSA 2013b and EFSA 2013c).

DOCUMENTATION PROVIDED TO EFSA

1. FERA (UK Food and Environment Research Agency):"Effects of neonicotinoid seed treatments on bumble bee colonies under field conditions". March, 2013. Authors: Helen Thompson, Paul Harrington, Selwyn Wilkins, Stephane Pietravalle, Dinah Sweet and Ainsley Jones.

2. Raw data to the study report "Effects of neonicotinoid seed treatments on bumble bee colonies under field conditions" (March, 2013; H. Thompson *et al.*): Colony weights, Flights activity, Residue data. Submitted by H. Thompson in April 2013 at the request of EFSA.

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APPENDIX

A critical evaluation of the statistical analyses in relation to the interpretation of the biological results

EVALUATION

1. Introduction

The aim of this work is to provide an in-depth evaluation of the study ("Effects of neonicotinoid seed treatments on bumble bee colonies under field conditions"), particularly focusing on the statistical methodology adopted. The study under evaluation was conducted and published by the Food and Environment Research Agency (FERA).

2. Data

The data in the report are presented in the Appendix of the study report. In addition, EFSA requested the raw data from the authors, which were provided timely. However, considering the qualitative weaknesses of the study (see following sections), an in-depth re-analysis of the data was not considered as a necessary step.

3. Material and Methods

3.1 Study design

3.1.1 Hypothesis testing

The study was conceived and set up in a hypothesis testing framework formally summarised as follows:

- *Null Hypothesis (H_0)*: exposure of bumble bee colonies to neonicotinoids leads to major effects on the colonies health status.
- *Alternative hypothesis(H_1)*: exposure of bumble bee colonies to neonicotinoids does not lead to major effects on the colonies health status.

Considerations

- ✓ The definition of "major effect" is not given.
- ✓ The aim of the study changed *in itinere* and was formulated as follows: "The objective was to examine the effects on bumble bee colonies in conditions as close as possible to real-life conditions". Of course, this objective (descriptive study, cross-sectional) is completely different from the original one and requires other methodological approaches than the one illustrated in the FERA study.
- ✓ There is an additional possible objective of the study (see Section 5 "Discussion" of the FERA report): it is stated that "the study has shown that bumble bee colonies remained viable and productive in the presence of neonicotinoids pesticides under these field conditions". Still, this sentence does not help in clarifying the meaning of "major effect".

3.1.2 Sample Size and Power calculation

- No sample size calculation is presented.
- No Power calculation was performed at the study design stage.

- Considering the available information from the scientific literature and previous EFSA publications on the variability to be included in these kind of studies, the sample size appears inadequate to detect any difference (with the exception of a severe effect, *i.e*. the death of all bees in a colony).

Considerations

- ✓ The absence of a Power calculation and a sample size calculation is probably linked to the missing definition of "major effect" (see section 3.1.1).

- ✓ Some indications on the sample size calculation are given. As an example, with a coefficient of variation between colonies of 15 % (σ^2=0.022), a coefficient of variation between fields of 5 % (σ^2=0.0025), and a number of colonies per field equal to 20 (as in the FERA study), the number of fields needed is 24 (8 for each group, *i.e.* 1 group of 8 control fields, 1 group of 8 treatment "B " fields and 1 group of 8 treatment "C" fields; total number of colonies = 480).

- ✓ In Thompson *et al.* (2013), another issue that needs to be taken into account is the multiple testing: many parameters are evaluated and tested to highlight differences between colonies. The α value should have been corrected accordingly (EFSA, 2011).

3.1.3 Treatment fields and control fields

- The design of the study included 3 different types of fields: an untreated field, a field treated with clothianidin and a field treated with imidacloprid.

Considerations

- ✓ The analysis of the residues revealed that there was no difference between the control field colonies and the two treated fields colonies, *i.e.* the colonies placed in the control field were in fact exposed to thiamethoxam and clothianidin. More precisely, the difference between the 3 groups of colonies was based only on the type of pesticide and its related level, but no group could be defined as "control" anymore, as none of them were really free from pesticides. This was probably due to the fact that the workers went foraging over the borders of the field of interest, bringing to the colony pollen and nectar other than that from the treated crops. In this situation, the original scope of the study is heavily compromised as the comparison has no term of reference anymore (parameter values under non-treatment conditions). In other terms, as all colonies were exposed to some neonicotinoids, no difference between groups can be really expected, but the one possibly linked to the level of exposure (which can be different across the 3 groups). However, all the considerations on the sample size remain valid also in this case.

- ✓ In addition, the exposure assessment performed on the colonies located in the treated fields revealed that the colonies were exposed to different neonicotinoids and not only to the one foreseen at the design stage. Again, this inconvenience makes it difficult to expect some difference between the groups.

- ✓ Referring to the previous bullet point, an analysis on the actual difference between exposure (based on the residue analysis) could be useful in the interpretation of the results (see section 3.2.2), *i.e*. if there is no significant difference in terms of exposure, it is unlikely to find any difference in the related colonies.

3.1.4 Random allocation

- The bumble bee colonies were randomly allocated to the three test sites.

Considerations

- ✓ The authors stated clearly that this was not exactly what happened as the colonies at test site C were assigned around 2 weeks later, therefore no randomisation was possible.

3.2 Analysis and results

3.2.1 Colony structure (page 17 of the FERA study)

- All the results on the parameters are reported together with the level of significance.

Considerations

- ✓ The data were correctly adjusted for the relevant initial values and consequently analysed. All the results are reported in the Result section (section 4 of the FERA report). However, it is not clear why Table 4 of the FERA report only shows the results of the analysis conducted on non-adjusted data. More emphasis should be given to the corrected values which, at a first glance, lead to much less significant results.

3.2.2 Residue analysis (page 22 of the FERA study)

- The results of the residue analyses performed were adequately reported for the purposes of statistical assessment.

Considerations

- ✓ No statistical test was performed to assess if the difference between exposure was significant or not.

3.2.3 Residue-based analysis (page 23 of the FERA study)

- Significant relationships were detected, but were considered artificial as driven by a few points with high leverage on the regression (outliers). Those values were then removed and the new analysis did not show any significant difference anymore.

Considerations

- ✓ The choice of removing data is not exhaustively supported by a full biological explanation.

- ✓ The meaning of the significant results, both using parametric and non-parametric tests, is not adequately discussed. Apparently, the relationship between thiamethoxam in pollen, thiamethoxam in nectar, clothianidin in nectar and colony mass cannot be really disregarded with ease.

- ✓ It is not clear why not all combinations are commented in the section (e.g. clothianidin in pollen, imidacloprid in nectar, etc.).

- ✓ It is not clear what is reported in Table 7 (page 24 of the FERA study). The problem arises when comparing line 2 of Table 7 (where the percentage of significant iterations is 0) and the following related paragraph (thiamethoxam in nectar).

3.3 Discussion

- It is clearly stated that "the study did not show conclusively that exposure to neonicotinoids used within normal agricultural setting had major effects on bumble bee colonies".

Considerations

- ✓ The statement does not specify that the study did not allow for any conclusion also on the alternative, *i.e.* there is no evidence that exposure to neonicotinoids used within normal agricultural setting had NO major effects on bumble bee colonies. More concisely, it can be stated that the study did not allow to draw any conclusion on the effects of neonicotinoids on exposed bumble bee colonies compared to non-exposed bumble bee colonies. The reason for this lack of significance is broadly discussed by the authors (short timeframe as primary cause).

4 Final considerations

The study has some points of strength. As an example, some aspects are highly appreciable in a controlled field trial:

- ✓ The use of standard starting colonies
- ✓ Random allocation for test sites A and B

However, considering the problems occurred and the weaknesses highlighted in the sections above, the study does not provide enough evidence to draw any conclusion on the effects of neonicotinoids on bumble bee colonies under field conditions. The authors themselves stated it clearly in the study report, specifying that more data and further research are needed.

RECOMMENDATIONS

Further studies should be conducted, with a higher level of control in the field, in order to assess the impact of neonicotinoids in bumble bee colonies under field conditions.

REFERENCES

Scientific Assessment Support (SAS) Internal Technical Report "Statistical questions related to Guidance document on Risk Assessment of Plant Protection Products on bees".

ABBREVIATIONS

µg	microgram
a.s.	active substance
FERA	Food and Environment Research Agency (UK)
g	gram
GAP	good agricultural practice
ha	hectare
kg	kilogram
L	litre
LOD	limit of detection
LOQ	limit of quantification
mg	milligram