Department for Environment, Food and Rural Affairs

Neonicotinoid insecticides and bees

The state of the science and the regulatory response

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Contents

The issue....1

Conclusions....1

The nature of the concerns raised....2

The latest studies....2

Defra's approach to this issue....3

Outcomes of consideration of recent research....3

Filling the evidence gaps....5

Developments in Europe....5

Annex 1: Recently published research on neonicotinoid insecticides....7

1. A common pesticide decreases foraging success and survival in honey bees....7

2. Neonicotinoid pesticide reduces bumble bee colony growth and queen production 8

3. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*....10

4. *In situ* replication of honey bee colony collapse disorder....12

5. Exposure to sub-lethal doses of fipronil and thiacloprid highly increases mortality of honey bees previously infected by *Nosema ceranae*....15

6. Dietary traces of neonicotinoid pesticides as a cause of population declines in honey bees: an evaluation by Hill's epidemiological criteria....16

7. Honey bees (*Apis mellifera*) reared in brood combs containing high levels of pesticide residues exhibit increased susceptibility to *Nosema* (Microsporidia) infection.20

8. Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behaviour.....21

9. Assessment of the environmental exposure of honey bees to particulate matter containing neonicotinoid insecticides coming from corn coated seeds 22

10. Ecological appropriate xenobiotics induce cytochrome P450s in *Apis mellifera* ...23

11. RFID Tracking of Sub-lethal Effects of Two Neonicotinoid Insecticides on the Foraging Behavior of *Apis mellifera* 24

12. The potential impacts of insecticides on the life history traits of bees and the consequences for pollination.....25

13. Using video-tracking to assess sub-lethal effects of pesticides on honey bees (Apis mellifera L.) 25

14. Parasite-insecticide interactions: a case study of *Nosema ceranae* and fipronil synergy on honey bee.....27

15. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment.....29

Annex 2: The regulation of pesticides.....31

Annex 3: Honey bee risk assessment under EU pesticide regulations.....32

For pesticides that are applied as a *spray*.....32

For pesticides that are applied as *seed treatments* or as a *solid* formulation.....33

Development of the risk assessment 33

Annex 4: ACP advice: neonicotinoids and bees 34

The issue

1. Several scientific studies published earlier this year suggest that low doses of neonicotinoid insecticides can have sub-lethal effects on bees with consequences for bee populations. Defra takes such suggestions very seriously and has therefore been considering these studies, alongside other evidence, to consider whether:

- further work is needed to extend our knowledge;
- there is a need to develop the way in which the effects of pesticides on bees are assessed; or
- further restrictions on the use of neonicotinoids are required.

Conclusions

2. The new research has been considered alongside existing knowledge, including the studies submitted to support current regulatory approvals for the neonicotinoids. This work has been carried out by Government and independent experts, taking account of parallel work in Europe. The broad conclusions of this work are as follows:

- Some of the new studies provide evidence of sub-lethal effects of neonicotinoids in the conditions applied in the research.

- However, none of the studies gives unequivocal evidence that sub-lethal effects with serious implications for colonies are likely to arise from current uses of neonicotinoids.

- Existing studies submitted in support of the present regulatory approvals fully meet current standards. They do not explicitly address all the sub-lethal effects suggested by the academic research. However, they do cover a wide range of important endpoints and, in these studies, hives exposed to treated crops did not show any gross effects when compared to control hives exposed to untreated crops.

Based on these findings, Defra has concluded that:

- It is appropriate to update the process for assessing the risks of pesticides to bees in the light of developments in the science - including the latest research. This exercise should include the development of a new risk assessment for bumble bees and solitary bees, alongside an updated risk assessment for honey bees. This work is being taken forward in Europe and UK experts are active in this. The aim is to complete this highly complex task by the end of 2012.

- Further research will be carried out to fill identified evidence gaps, including the questions raised about the relevance of the recent studies to field conditions. The Government has already put new research in place to explore further the impacts of neonicotinoids on bumble bees in field conditions and to understand what levels of pesticide residues and disease in bees are normal.

- The recent studies do not justify changing existing regulation. However, the research that we have put in hand and the on-going work in Europe to develop the risk assessment could change the picture and it is always possible that further new evidence may emerge. As our knowledge develops, we will continue to consider the need for further research and for any changes to the regulation of neonicotinoids.

The nature of the concerns raised

3. Bees are important, not least for their role as pollinators. Over recent years there have been concerns raised about rates of colony losses for honey bees and declines in populations of bumble bees and solitary bees. The picture is complicated and the evidence suggests that bee health is influenced by a number of factors – particularly pests and pathogens, husbandry (in the case of honeybees), nutrition and the weather.

4. Another factor that has been suggested as impacting on bees is the use of pesticides in general and neonicotinoids in particular. There are five neonicotinoid active substances authorised for use in the EU – acetamiprid, clothianidin, imidacloprid, thiacloprid and thiamethoxam – which are found in insecticides widely used to protect a range of crops from aphids and other pests. This use of neonicotinoids began in the UK in the late 1990s and has grown steadily.

5. Over recent years, a number of studies have suggested that, depending on the exposure level, neonicotinoids may have adverse effects on bees – both honey bees and bumble bees. The suggestion is that the effects are sub-lethal but cause sufficient disruption to the normal functioning of bees to be a threat at the colony or population level.

The latest studies

6. A number of further studies have been published in recent months and are summarised in **Annex 1**. The four studies which have received the most publicity are:

- Henry *et al* "A common pesticide decreases foraging success and survival in honey bees", published in Sciencexpress on 29 March 2012

- Whitehorn *et al* "Neonicotinoid pesticide reduces bumble bee colony growth and queen production", published in Sciencexpress on 29 March 2012

- Pettis *et al*, "Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*", published in Naturwissenschaften, February 2012

- Lu *et al* "In situ replication of honey bee colony collapse disorder", published in the Bulletin of Insectology, June 2012.

Defra's approach to this issue

7. Pesticides, including neonicotinoids, are regulated under strict EU rules and can only be sold or used if they are approved. Approval is only granted if assessment of scientific data shows that risks are acceptably low. The current risk assessment addresses risks to honey bees and to two other non-target arthropods but not, specifically, risks to other bee species. More details of the regulatory system are at **Annex 2**; the risk assessment approach for honey bees is outlined in **Annex 3**. Conditions are routinely attached to approval (for example specifying dose rates, timing and place of application) to ensure protection of human health and the environment (including wildlife). Approvals are regularly reviewed (usually every ten years) to ensure that they continue to meet current standards. All the neonicotinoids have been individually assessed and approved under the requirements of the EU regime.

8. These regulatory controls on pesticides are strong but the Government is not complacent and takes very seriously any threat to bees and other pollinators. Defra therefore looks very carefully, and with an open mind, at the developing evidence. We will not hesitate to take any action that proves to be necessary. This could include restricting or withdrawing product authorisations; such measures have been taken in previous cases when found to be necessary.

9. Accordingly, the recent studies and existing evidence have been assessed by: the Chemicals Regulation Directorate (CRD) of HSE; bee experts in Defra's Food and Environment Research Agency (Fera); Defra's Science Advisory Council; and the independent expert Advisory Committee on Pesticides (ACP). Outcomes of this work are reported below. UK experts have also been involved in work carried out by the European Food Safety Authority (EFSA) (also reported below) and have drawn on this in their own consideration. Alongside the consideration of the new studies, work has also been put in hand (see paragraph 17) to fill several evidence gaps that have been identified.

Outcomes of consideration of recent research

(i) Consideration by CRD and Fera

10. CRD and Fera views are outlined in **Annex 1**. On the papers listed in paragraph 6 above, the key observations are as follows:

- The Henry *et al* study provides information regarding the potential adverse effects of thiamethoxam on the foraging behaviour and resulting survival of honey bees. Due to the artificiality of the test design and dosing regime, there are uncertainties regarding the risk in a more realistic field exposure situation. As part of the regulatory assessment, an extensive dataset on the potential effects of thiamethoxam on honey bees when used as a seed treatment on oilseed rape was considered. This included multi-year / multi-site field trials which indicated an acceptable risk.

- As bumble bees are not considered under current EU pesticides law (see the future plans at paragraph 18 below), it is more difficult to assess the significance of the findings of the Whitehorn *et al* study. It may be significant that the control bees

consumed nectar and pollen whereas the treatment bees were given a different diet of treated pollen and sugar water. The key question for this study is how far it illuminates the likely real situation at field level - are the exposure and the resulting effects seen under normal conditions?

- Taking the Pettis *et al* study at face value it can be concluded that exposure to imidacloprid may result in higher levels of Nosema. The following points require consideration in trying to interpret this study: whether factors such as exposure are in line with field situations; the significance at the colony level; the variability of *Nosema* spore count appears to be high; and the use of bulked and uneven samples. In order to determine if there is a real concern regarding the risk to honey bees that may be infected with *Nosema* from the consumption of pollen/nectar treated food, it would be necessary to carry out studies under more realistic conditions.

- The Lu *et al* study does not raise new concerns in respect of the impact of neonicotinoids on bees. The doses chosen are unrealistically high for exposure of bees from treated flowering crops; the food source chosen is not in significant use in the UK and the residues used are not related to any assessment of those actually present; and it is not clear whether the effects seen are in fact similar to those of Colony Collapse Disorder (which is, in any case, not encountered in the UK).

11. CRD's overall conclusion was that the studies identify issues but do not justify the imposition of further restrictions on neonicotinoids at this stage. Rather, the findings to date call for continuing investigation and the development of the regulatory risk assessment process. CRD's analysis was fed into the subsequent examination by the ACP.

(ii) Consideration by the ACP

12. The ACP considered the issue at its meetings on 15 May and 3 July. The recommendations agreed following the 3 July meeting are set out in full at **Annex 4**. In summary, the ACP has concluded that the current UK risk assessments are secure and recommended that there is no justification for regulatory action at present. Furthermore, there is no evidence as yet of neonicotinoid impacts on bees in the UK. However, the ACP will consider any new information as it arises and keep the situation under close review. The Committee supports the evidence gathering and development of the risk assessment that is in hand here and in Europe.

13. The ACP's conclusion was based on reconsideration of studies supporting the current authorisations for thiomethoxam products and on detailed examination of the recent publications in the scientific literature, with one of the ACP's ecotoxicology experts carrying out a careful examination of the raw data.

14. The regulatory field studies fully comply with current guidance and also cover some additional aspects, such as over-wintering. The power of the studies to detect statistically significant changes is not established and they would not show all of the specific sub-lethal effects suggested by academic studies. However, hives exposed to treated crops did not

show any gross effects on a wide range of important endpoints when compared to control hives exposed to untreated crops.

15. While noting questions concerning aspects of the two published studies (by Henry et al and Whitehorn et al), the ACP does not discount their findings. The Committee believe these studies should be considered in the development of future regulatory guidance. Further research is merited to clarify the findings and their relevance to the UK field situation. The ACP noted that relevant work is already being taken forward with urgency. The Committee will keep this research, and its potential implications for authorisations, under review.

16. The ACP identified other possible areas for research, including work on bee toxicokinetics to examine factors related to dose and exposure period and a true field study looking at disorientation (while recognising the very real difficulties in successfully conducting such a project). The ACP also asked its Environmental Panel to look at recently completed work on guttation (the exudation of xylem sap from vascular plants) as a potential source of exposure to other non-target arthropods.

Filling the evidence gaps

17. Defra has carried out R&D around these issues over a number of years. In the light of the new studies, two further projects have been commissioned from Fera and are due to be completed by March 2013:

- The first (PS2370) will focus on the interpretation of pesticide residues and disease in honey bees. Dead bees are sometimes sent in as part of a wildlife incident. These are routinely screened for pesticides and low levels of pesticides are often found which are unlikely to have been the cause of death. This new research will help us interpret the wildlife incident results by obtaining some apparently “healthy” bee samples from the bee inspectors own bee hives and analysing them for pesticide residues and for disease levels. The hives will also be looked at next year to ensure that the bees survived the winter.

- The second (PS2371) is designed to explore the findings of the Whitehorn *et al* study, using more realistic conditions. It is looking at real life edge of field exposure of bumble bees to neonicotinoid treated flowering oilseed rape (both spring sown and winter sown).

Developments in Europe

18. Pesticide regulation is harmonised across Europe. The European Food Safety Authority (EFSA) is carrying out a number of pieces of work (in which UK experts are involved) including:

- EFSA’s Panel on Plant Protection Products and their Residues published a Scientific Opinion on the science behind the development of a pesticide risk assessment for honey bees, bumble bees and solitary bees on 23 May. This is available at http://www.efsa.europa.eu/en/efsajournal/doc/2668.pdf and is a very substantial and significant review and analysis of the state of the science.

- The Opinion will be the basis for a full revision of the rules for the risk assessment of pesticide impacts on bees, including the development of a new risk assessment process for bumble bees and solitary bees. A new guidance document is due to be drawn up by the end of December.

- EFSA published a Statement on 1 June addressing the significance of the Henry *et al* and Whitehorn *et al* studies. This Statement is available at: http://www.efsa.europa.eu/en/efsajournal/doc/2752.pdf. In brief, their findings were:

  Comparing the Henry *et al* study with possible real life exposures, EFSA conclude that sub-lethal effects cannot be fully excluded in worst case situations. However, they note several uncertainties regarding the results**.** In particular, in the study, bees consumed the total amount of active substance within a relatively short period rather than during the course of a day. Depending on the substance properties and how fast the substance can be metabolised by the bees, this method of exposure could lead to more severe effects than may occur when bees are foraging.

  The concentrations tested on bumblebees by Whitehorn *et al*. were in the range of the maximum plausible exposure levels from imidacloprid in pollen and nectar. However, it is uncertain as to what extent the 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.

- EFSA are reviewing the current data relating to bees for the three neonicotinoid active substances that have high acute toxicity to bees; this work is due to be completed by the end of 2012.

Annex 1: Recently published research on neonicotinoid insecticides

1. A common pesticide decreases foraging success and survival in honey bees

Authors: Mickaël Henry, Maxime Beguin, Fabrice Requier, Orianne Rollin, Jean François Odoux, Pierrick Aupinel, Jean Aptel, Sylvie Tchamitchian and Axel Decourtye

Published: Sciencexpress/29 March 2012/Page 10.1126/science.1215039

Summary

The study tested the hypothesis that a sub-lethal exposure to a neonicotinoid indirectly increases hive death rate through homing failure in foraging honey bees. The study used thiamethoxam and involved two phases: an assessment of mortality induced by homing failure in exposed foragers; and an assessment of the extent to which homing failure in combination with natural forager mortality could upset colony dynamics.

A total of 653 free-ranging foragers were fitted with radio-frequency identification devices (RFID). In order to simulate exposure to thiamethoxam, foragers were given 1.34 ng in 20-µL sucrose solution. The bees were then released away from their colony. RFID readers were placed at the hive entrance. Mortality was then determined as the proportion of non-returning bees. Control bees were fed sucrose only and released in the same way. Tagged honey bees were released up to 1 km away from their respective colony. Experiments were conducted on individuals from three different colonies.

To account for individuals' past foraging experience, two distinct homing experiments were carried out. Experiment 1 simulated intoxication at a familiar foraging site whilst experiment 2 involved a random site. Experiment 1, used 'familiar foragers', i.e. those foragers which had covered at least once the pathway from the release site back to the colony. Bees with pollen loads of *Phacelia* were used to identify those bees that had a known pathway.

For experiment 2, bees with no *Phacelia* pollen loads were used, i.e. it was assumed that these did not have a known pathway. All bees were released in a 1 km circle around the colony.

Both experiment 1 and 2 evidenced substantial mortality due to post-exposure homing failure, with the proportion of treated foragers returning to the colony being significantly lower than that of control foragers. Post exposure homing failure was greater in treated

foragers that tended to be unfamiliar with the foraging site, as indicated by their significantly lower homing proportions compared to familiar foragers.

On the basis of the results from experiment 1 (bees with a known pathway) the authors propose that 10.2% of exposed honey bees would fail to return to their colony after foraging in a treated field, whilst for those bees with no known pathway 31.6% would fail to return. The study authors state that the probability that a foraging bee dies naturally on a particular day is 15.4% (simply based on an average lifespan for foragers of 6.5 days). The study authors use this information in a honey bee population model and predict that there would be an impact on the size of the colony to a level rarely seen in current beekeeping practices.

Two further experiments were done. In one, bees were released 70 m away rather than 1 km; the homing failure was much reduced but still significant. In the other, the authors repeated experiment 2 in a different landscape. A beehive was placed in a suburban area in southern France, which included a mosaic of mixed farming fields and orchards of moderate size. Foragers were released 1 km away at six equidistant sites. Homing failure was significant (9.8%) but much smaller than in experiment 2 (31.6%).

CRD view

This study provides some interesting information regarding the potential adverse effects of thiamethoxam on the foraging behaviour and resulting survival of honey bees. Due to the potential artificiality of the test design and dosing regime compared to exposure under field conditions, there are uncertainties regarding the interpretation of this study, in particular what is the risk under a more realistic exposure situation?

Thiamethoxam is used in the UK as a seed treatment on oilseed rape as well as other crops. As part of the regulatory assessment, an extensive dataset on the potential effects of thiamethoxam on honey bees when used as a seed treatment on oilseed rape has been considered. This dataset includes multi-year and multi-site field trials which assess the risk to honey bees from foraging oilseed rape flowers grown from treated seed. These data indicate an acceptable risk.

EFSA published a Statement on 1 June addressing the significance of this study. This Statement is available at: http://www.efsa.europa.eu/en/efsajournal/doc/2752.pdf.

2. Neonicotinoid pesticide reduces bumble bee colony growth and queen production

Authors: Penelope R. Whitehorn, Stephanie O'Connor, Felix L. Wackers, Dave Goulson

Published: Sciencexpress/ 29 March 2012/Page1/10.1126/science.1215025

Summary

A study using 75 *Bombus terrestris* colonies was carried out to simulate the likely effect of exposure of a wild bumble bee colony to imidacloprid present on the flowers of a nearby treated crop. Control colonies received *ad lib* pollen and nectar over a period of 14 days in the laboratory. Colonies in the low treatment regime received 6 and 0.7 µg/kg in pollen and sugar water respectively, whilst colonies in the high treatment regime received double these concentrations. The study author states that these concentrations had been based on measured concentrations from previously published work.

After two weeks of exposure all colonies were then placed in the field where they were left to forage independently for a period of six weeks. The field site was situated on the edge of Stirling University campus and close to ornamental gardens, deciduous woodland and mixed farmland, so that scattered patches of wild and ornamental flowers were available within foraging range. Colonies were randomly allocated to locations and evenly distributed across the site. There were no flowering crops within 2 km. It was noted that the exposure period in the laboratory of two weeks is less than the duration of flowering of oilseed rape in the field.

Colonies subjected to both the low and high treatments gained less weight over the course of the study than did the control colonies. The weight change in the high treatment was not significantly different from the low treatment colonies. By the end of the experiment the low and high treatment colonies were on average 8 and 12% smaller than control colonies.

No significant difference between treatments were found in the numbers of males, workers, pupae or empty cells at the end of the experiment, although the number of empty pupal cells was 18 and 30% lower in the low and high treatments respectively compared to the control. The mean number of queens produced by colonies in the control treatment was 13.72, whilst in the low and high treatment it was 2 and 1.4 respectively. The study authors' states that the decline in queen production is disproportionately large compare to the impact on colony growth.

CRD views

Bumble bees are not considered under the EU authorisations Regulation and hence it is more difficult to assess the significance of the findings of this study. The paper does raise potential concerns. It may be significant that the control bees consumed nectar and pollen whereas the treatment bees were given a different diet of treated pollen and sugar water. The relevance of this is unknown.

The key question for this study is how far it illuminates the likely real situation at the field level. For example are the exposure and the resulting effects seen under normal conditions?

As a result of this study, Defra is funding a project (PS 2371) examining the potential effects of imidacloprid on bumble bees foraging oilseed rape grown from imidacloprid treated seed under field conditions.

EFSA published a Statement on 1 June addressing the significance of this study. This Statement is available at: http://www.efsa.europa.eu/en/efsajournal/doc/2752.pdf.

3. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*.

Authors: Pettis J S, van Engelsdorp D, John J and Dively G

Published: Naturwissenschaften, 2012 Feb; 99(2): 153-8 Epub 2012 Jan 13.

Summary

This study exposed honey bee colonies during three brood generations to sub-lethal doses of imidacloprid, and then subsequently challenged newly emerged bees with the gut parasite, *Nosema* spp. Hence, the hypothesis was that bees exposed to sub-lethal levels of pesticide are more susceptible to disease. The pesticide dosages used were stated to be below levels demonstrated to cause effects on longevity or foraging in adult honey bees.

The method used was as follows: for 10 weeks, full sized colonies of bees (30–40,000 adults) were exposed to 5 and 20 ppb imidacloprid by provisioning colonies with protein supplement patties spiked with the pesticide. After 5 and 8 weeks of exposure (ca. 1.5 and 2.5 generations of exposure), wax combs with emerging brood were taken into the laboratory and groups of newly emerged adult bees from selected colonies were removed and either used to determine fresh weight or caged and fed a suspension containing spores of the known bee pathogens *N. apis* and *N. ceranae* over the first 2 days of adult life. Ten days later, bees were sacrificed and the development of *Nosema* infection in individual bees determined.

Key results were as follows:

- In both trials, bees originating from colonies feed high and low levels of imidacloprid had higher *Nosema* spore counts than controls.
- No difference was observed in the final spore counts in bees fed different doses of *Nosema* – hence data from the two doses of *Nosema* were combined.
- Residues of imidacloprid were found in bee bread and bees from exposed colonies and increased in direct and expected proportion to the concentrations in the treated protein patties. Traces of imidacloprid were also found in bees and bee bread collected from control colonies.

- Bees from the higher 20-ppb pesticide exposure colonies were significantly lighter in weight in the July trial. However, bees from the August trial were comparable to the control.

- The concentration of imidacloprid in protein patties fed to colonies did not have an effect on final *Nosema* spore counts in 12-day-old bees. Similarly, the dose of *Nosema* spores provided to emerged bees (0.1 or 1 million spores per ml of sucrose water) did not affect total spore count in 12-day-old bees.

At the end of 10 weeks, eight of 30 colonies tested positive for *Nosema* but there was no relationship between *Nosema* infection and imidacloprid treatment which would have been predicted by the lab study. Three control, three 5 ppb, and two 20 ppb colonies tested *Nosema* positive, with average spore counts of 4.3, 2.9, and 0.5 million spores per bee, respectively. This finding was described as 'surprising' by the study author.

CRD views

Taking the study at face value it can be concluded that exposure to imidacloprid may result in higher levels of Nosema compared to the controls. The following points require further consideration in trying to interpret this study:

- How does this study relate to the field situations? The concentrations of imidacloprid used were 5 and 20 ppb, the study author states that 'crop residues have detected imidacloprid at levels of 2-5 ppb in pollen and >1.5 ppb in nectar of seed-treated corn, sunflowers and rape. Therefore, exposure in the study is partially in line with exposure in the field'.

- The significance of the findings to likely colony effects was not considered.

- The variability of *Nosema* spore count appears to be high – the range of spore counts in 12-day-old bees in July ranged from slightly less than 0.2 $x10^6$ in the control to approximately 0.7 x 10^6 in both the low and high doses. In the August study, the control was 1.0 $x10^6$, whilst the low treatment was approximately 1.7 $x10^6$ and the high treatment was 1.5 $x10^6$. The paper is silent on this high variability between months. Also, the samples were bulked and the sample sizes were uneven. For example there were 30 bees from 3 colonies in the control in the July sample, compared to 20 bees from 2 colonies in the low and 40 bees from 4 colonies in the high.

The above points do not negate the study, however they raise questions – in particular point 2. In order to determine if there is a real concern regarding the risk to honey bees that may be infected with *Nosema* from the consumption of pollen/nectar treated food, it would be necessary to carry out studies under more realistic conditions. This may be possible by exposing infected bees under semi-field or field conditions. The current Fera project (PS2371) looking at real life exposure of bumble bees would provide some of the background to this.

4. *In situ* replication of honey bee colony collapse disorder

Authors: Lu C., Warchol K.M. and Callahan R.A.

Published: 13 March 2012 - corrected PROOF Bulletin of Insectology 65 (1): xxx-xxx, 2012 ISSN 1721-8861

Summary

The paper considers the potential role of neonicotinoids in colony collapse disorder or CCD. The paper defines CCD as 'the sudden disappearance of honey bees (specifically worker bees) from hives containing adequate food (e.g. honey, nectar, and pollen) and various stages of brood in abandoned colonies that are not robbed by honey bees from other colonies'. The paper cites the following as being potentially linked to CCD: *Varroa;* Israel acute paralysis virus; *Nosema ceranae;* and exposure to neonicotinoid insecticides. It also cites the practice of migratory commercial beekeeping and malnutrition associated with mono-cultural food sources as being potential causes of CCD.

The study hypothesis is that the first occurrence of CCD in 2006/7 resulted from the presence of imidacloprid in high-fructose corn syrup (HFCS) fed to honey bees as an alternative to sucrose-based food. This was based on the following three reasons:

- Most of the reasons cited above were not new to apiculture and therefore CCD must have been caused by a new factor.
- Take up of HFCS as a food supply
- Residues of imidacloprid in pollen from genetically engineered seeds treated with imidacloprid

The study design consisted of four sites 12 km apart. Each site had five hives placed next to an unspecified crop. The five hives consisted of – a control, four hives fed initially at 0.1 µg/kg, 1 µg/kg, 5 µg/kg and 10 µg/kg imidacloprid. The initial phase lasted for 4 weeks and started 1st July. The second phase involved treating the hives treated at 0.1 µg/kg, 1 µg/kg, 5 µg/kg and 10 µg/kg for 9 weeks at 20 µg/kg, 40 µg/kg, 200 µg/kg and 400 µg/kg respectively. The hives were monitored throughout the year (i.e. brood development was assessed) and then the hives were allowed to overwinter and their survival and overwintering success monitored.

The number of sealed brood for both treated and control hives decreased significantly from July to September, however this decrease was independent of the imidacloprid doses applied to the hives. All 20 hives were stated to be alive when they were assessed in December, i.e. 12 weeks post imidacloprid dosing (PID). The authors did state that the highest dose did appear to be weakening as observed by small clusters and frozen dead honey bees in front of the hive. Details of the study and in particular the occurrence of dead hives is presented below:

Table 3. The progression of the *in situ* study and the dates of dead honey bee hive observation.

Date	Event
Jan-Feb, 2010	Assembling 20 new 10-frame Langstroth pine honey bee hives.
March, 2010	Study site selection and apiary setup.
March 28th, 2010	Introducing honey bees (bee shaking) to 20 new hives in 4 apiaries.
May 21st, 2010	All 20 hives contained at least 15 frames of capped brood.
July 1st - 29th, 2010	Initial low imidacloprid dosing for 4 consecutive weeks.
July 29th - Sept 30th, 2010	Follow-up high imidacloprid dosing for 9 consecutive weeks.
July-Sept, 2010	Monitoring strength of honey bee hives biweekly.
Oct 5th - Nov 20th, 2010	Parasite treatment (Apistan strips and Fumagillin B) on all hives.
Dec 3rd, 2010 - present[1]	Winter hive strength monitoring.
Dec 22nd, 2010 - present[1]	Feeding hives with crystallized HFCS mixed with granular sucrose.
Dec 22nd, 2010	Last monitoring date without the observation of dead hives.
Dec 31st, 2010	The 1st and 2nd hives treated with 400 µg/kg imidacloprid dose dead.
Jan 7th, 2011	The 1st hive treated with 40 µg/kg imidacloprid dose dead.
Jan 14th, 2011	The 1st hive treated with 200 µg/kg imidacloprid dose dead.
Jan 19th, 2011	The 2nd hive treated with 200 µg/kg imidacloprid dose dead.
Feb 4th, 2011	The 3rd and 4th hives treated with 400 µg/kg imidacloprid dose dead. The 2nd hive treated with 40 µg/kg imidacloprid dose dead.
Feb 24th, 2011	The 3rd, 3rd and 4th, and 1st and 2nd hives treated with 200, 40, and 20 µg/kg imidacloprid dose, respectively dead. The 1st control hive dead.
March 10th, 2011	The 4th and 3rd hive treated with 200 and 20 µg/kg imidacloprid dose, respectively, dead. The 4th hive treated with 40 µg/kg imidacloprid and 3 control hives remain alive.

[1] On-going activities as of March 21st, 2011.

The following statements are made in the discussion:

The magnitude and the pattern of honey bee hive loss during the winter months in this study resemble the reported symptoms of CCD. The loss of 15 of 16 imidacloprid-treated hives (94%) across 4 apiaries occurred over a period of 10 weeks following the first hive death. Dead hives were remarkably empty except for stores of food and some pollen left on the frames.

Although this observation is not quite reminiscent of the reported CCD symptoms, it is important to consider that if these hives were located in a warmer climate region, such as in Florida USA where migratory hives overwinter, bees exiting the hives would have dispersed some distance from the hives and therefore would not be observed in front of the hives.

It is interesting to note that the symptoms observed are not in line with authors' definition of CCD, however they still refer to the effects being in line with CCD. It is also interesting to note that the authors state 'all hives were considered healthy as they went into fall season' when earlier in the text they highlight that the bees exposed to the highest concentration were already dying.

The authors state that the concentrations were not only environmentally relevant, but also lie within legally allowable levels, set by the US Environmental Protection Agency (EPA). This level is 0.05 ppm (50 µg/kg) for corn. As there is no tolerance level for imidacloprid in HFCS, the study authors applied a 10-fold concentrating factor, or 0.5 ppm (500 µg/kg) of imidacloprid in HFCS. The authors consider that the 10-fold concentrating factor is 'very conservative compared to the reported average level of 47 mg/L of imidacloprid measured in guttation drops collected from corn seedlings germinated from commercial seeds obtained in 2008 coated with 0.5 mg/seed of imidacloprid'. No reference is made to the likely residues in HFCS made from corn grown from treated seed.

CRD views
As with all studies of this sort, the level of exposure is key and knowing that the concentration is in line with that likely to be encountered in an agricultural crop is essential. In this case, whilst the study's authors indicate that the concentrations are realistic and environmentally relevant, this does not appear to be the case.

It should be noted that the diet fed to bees is not normally looked at as part of the risk assessment and therefore there is a lack of data in this area. There is generally a lack of data on the residue levels in pollen, nectar and the diet fed to bees when over-wintering and when bees are fed at other times when they are unable to forage.

However, there are several relevant studies. A study on the levels of imidacloprid in pollen and nectar taken from oilseed rape grown from treated seed found residues of up to 7.6 µg/kg in pollen; no residues were detected in nectar. This study used seed that had been treated at five times the rate used in the UK. Other studies suggest that realistic residues may be in the region of 2 to 10 µg/kg.

The range of concentrations used in the initial phase of the Lu *et al* study were 0.1 µg/kg, 1 µg/kg, 5 µg/kg and 10 µg/kg and exposure was for 5 weeks. The bees were then exposed to concentrations of 20 µg/kg, 40 µg/kg, 200 µg/kg and 400 µg/kg for a further 9 weeks. Whilst the initial phase is potentially in line with residues that may be encountered by bees foraging oilseed rape, the latter phase would appear to be clearly in excess of that likely to be encountered. It can therefore be concluded that, on the basis of the residue data we have, the exposures used were unrealistically high. It should also be noted that the exposure was much longer, and at a different time of year, than would be the case for exposure to a flowering crop.

The study author states that the second phase concentrations were partly based on what is permitted (not what occurs) in High Fructose Corn Syrup (HFCS) in the US.
It would have been more relevant to determine residues in commercially available HFCS and then use those figures as the dosing levels actually used may not even represent the levels of imidacloprid usually found in this food source. In any case, it is understood that there is minimal use of HFCS for feeding bees in the UK, hence further reducing the relevance of this study to the UK.

The effects seen are not in line with the authors' own definition of CCD. It is therefore unclear whether the effects seen can be classified as CCD or simply as colonies that has failed to make it through the winter. Various comments along similar lines have been made on the web.

Finally, it should be noted that the issue of overwinter survival looked at in this study was raised in the ACP's consideration of the Buglife report. The ACP agreed that studies were needed to address long-term effects and, in particular, overwintering success. This data requirement will be part of the regulatory system from next year.

In summary, this study does not raise new concerns in respect of the impact of neonicotinoids on bees. The reasons for this conclusion include:

(a) the doses chosen appear unrealistically high for exposure of bees from treated flowering crops;

(b) the food source chosen (HFCS) is not in significant use in the UK and the residues used are not related to any assessment of those actually present;

(c) it is not clear whether the effects seen are in fact similar to those of Colony Collapse Disorder (which is, in any case, not encountered in the UK).

5. Exposure to sub-lethal doses of fipronil and thiacloprid highly increases mortality of honey bees previously infected by *Nosema ceranae*.

Authors: Vidau C., Diogon M., Aufauvre J., Fontbonne R., Vigues B., Brunet J-L., Texier C., Biron D.G., Blot N., El Alaoui H., Belzunces L.P., Delbac F.

Published: PloS ONE 6(6): e21550. Doi 10.1371/journal.pone.0021550.

Summary

In this study newly emerged honey bees were divided in 6 experimental groups:

1. uninfected controls,
2. infected with *N. ceranae*,
3. uninfected and exposed to fipronil,
4. uninfected and exposed to thiacloprid,
5. infected with *N. ceranae* and exposed 10 days post-infection (p.i.) to fipronil, and
6. infected with *N. ceranae* and exposed 10 days p.i. to thiacloprid.

Honey bee mortality and insecticide consumption were analyzed daily and the intestinal spore content was evaluated 20 days after infection.

The key findings were:

1. Honey bees infected with *N. ceranae* consumed significantly more sucrose than uninfected honey bees.
2. Exposure to fipronil and thiacloprid had no effect on the mortality of uninfected honey bees compared to the control.
3. Honey bees infected with *N. ceranae* and then exposed to thiacloprid/fipronil died earlier than bees only infected with *N. ceranae.*
4. A significant increase in honey bee mortality was observed when *N. ceranae*-infected honey bees were exposed to sub lethal doses of insecticides. Mortality of

infected only bees was 47% compared to 82% and 71% for infected bees exposed to fipronil and thiacloprid respectively. Mortality of bees exposed to only thiacloprid or fipronil was equivalent to the control and was approximately 10%.

5. Statistical analysis showed that exposure to fipronil significantly reduced the microsporidian spore production in infected bees whereas exposure to thiacloprid significantly enhanced spore production.
6. Analysis of the honey bee detoxification system 10 days p.i. showed that *N. ceranae* infection induced an increase in glutathione-S-transferase activity in midgut and fat body but not in 7-ethoxycoumarin-O-deethylase activity, i.e. the synergistic effect of *N. ceranae* and insecticides on honey bee mortality did not appear strongly related to a decrease in the insect detoxification system.

CRD views

The study appears to be well conducted and clearly reported. It indicates synergistic effects of fipronil/thiacloprid and *N. ceranae*. The insecticide doses used were stated to be 1/100th of the oral LD50. The key issue is how this relates to potential exposure under field conditions – i.e. will bees be exposed to this level of pesticide under field conditions? No data were provided in the study report to indicate the relevance of this concentration. As part of the study bees were infected with *N. ceranae*; it is unclear how the level of infection related to the field situation or likely infection levels.

The lack of information on the relevance of dosing both with insecticide as well as *N ceranae* are not criticisms of the study. The study indicates that mortality was greater when *N. ceranae*-infected honey bees were exposed to doses of fipronil/thiacloprid under laboratory conditions.

6. Dietary traces of neonicotinoid pesticides as a cause of population declines in honey bees: an evaluation by Hill's epidemiological criteria

Authors: Cresswell J.E., Desneux N and van Engelsdorp

Published: Accepted article doi: 10.1002/ps.3290. Pest Management Science (2012)

Summary

The paper employs Hill's epidemiological 'causality criteria' as a structured process for making an expert judgement about the proposition that trace dietary neonicotinoids in nectar and pollen cause population declines in honey bees.

Cresswell *et al* states that the EU Regulation 1107/2009 does not require that pesticides are ecologically harmless, but instead specifies that member states may not authorize a crop protection product unless it has no unacceptable effect on the environment, including non-target species. Cresswell *et al*, rightly assumes that a pesticide's use is unacceptable if it seriously threatens a non-target species that contributes to human wellbeing by

delivering an important ecosystem service. The ecosystem service that Cresswell *et al* considers is the role that honey bees have in ensuring that crops are pollinated. The paper focuses on imidacloprid, which is widely used and studied, and on which there is plenty of available information. The paper cites three separate modes of exposure for bees: (i) direct exposure by dispersal in particulate clouds during seed drilling; (ii) oral ingestion of residues in guttation fluid of seedling maize; and (iii) trace dietary residues in nectar and pollen. However, the paper only concentrates on the latter mode only, i.e. the potential impacts of trace dietary residues in nectar and pollen.

The paper uses Hill's causality criteria along with certainty scores to each criteria to determine whether the available evidence supports the hypothesis that neonicotinoids cause honey bee declines. The criteria and a brief description are presented below:

Number	**Criterion**	**Brief description**
1	Experimental evidence	
2	Coherence	Fails to contradict established knowledge
3	Plausibility	Probable given established knowledge
4	Analogy	Similar examples known
5	Temporality	Cause precedes effect
6	Consistency	Cause is widely associated with effect
7	Specificity	Cause is uniquely associated with effect
8	Biological gradient	Monotonic dose-response relationship
9	Strength	Cause is associated with a substantive effect

To produce a quantitative score of certainty for each criterion, a range of descriptors are used to describe the level of conviction with which an evaluator holds a cause-effect hypothesis to be true: slight; reasonable; substantial; clear; and certain. These descriptors were associated with numerical values to create an eleven point scale for each criterion that returns a positive value (maximum five) if the evidence suggests that the factor (trace dietary neonicotinoid) certainly causes population decline, a negative value (maximum minus five) if the factor certainly does not and zero if the evidence is equivocal or lacking. For example, if the evidence for *i*th criterion gives a reasonable indication that neonicotinoids do not cause population declines in honey bees, the score for that criterion would be Ci = - 2, etc.

Understandably, the paper does not provide an exhaustive review of evidence however this does mean that it is difficult to determine what has and hasn't been considered and what conclusion has been reached on the available data; similarly it is clear that the authors have not had an opportunity to consider all the data produced for regulatory purposes.

The paper focuses exclusively on the proposition that neonicotinoid pesticides are capable in their own right of causing population declines in honey bees. This is due to the fact that the more complex hypothesis that neonicotinoids act in concert with other stressors needs to be considered only once the simpler case is dismissed.

Outlined below is a summary of each of the above criteria:

Experimental evidence – Cresswell *et al* concludes that 'experimental evidence to date has not demonstrated that trace dietary imidacloprid causes population decline, but neither has the testing been stringent enough under environmentally-relevant conditions to reject this causal hypothesis convincingly because of shortcomings in statistical power'. The hypothesis is, however, sustained to some degree by the sub-lethal, harmful effects that are detected in laboratory tests. Taking into account the limitations of field trials (statistical power, use of proxy response variables), Cresswell *et al* takes their null results as only a slight indication that neonicotinoids are not a cause of bee population decline and as a result scores this criterion as C1 = -1.

Coherence – Cresswell *et al* states that it is not possible to identify any conflict between existing knowledge and the proposition that neonicotinoids cause honey bee declines, but the quantitative shortcomings in current knowledge mean that this coherence provides only a substantial indication in favour of the proposition and this criterion was scored +3.

Plausibility – It was concluded that the proposition that trace dietary neonicotinoids cause honey bee declines is only reasonably plausible and as a result this criterion was scored +2.

Analogy – The analogy criterion asks whether a judgement can be supported by an appeal to similar, well-resolved cases. Cresswell *et al* concludes that the available analogies provide substantial evidence that trace dietary neonicotinoids could detrimentally affect vital demographic rates in honey bees and as a result this criterion scores +3.

Temporality – The temporality criterion asks whether the putative cause precedes the consequence – available evidence indicates that trace dietary neonicotinoids clearly neither preceded not apparently intensified the honey bee decline and as result the criterion was scored -4.

Consistency – the consistency criterion asks whether the association between the putative cause and it consequences is repeated in space and time. Cresswell *et al*

concluded that dietary neonicotinoids are clearly inconsistently associated with honey bee decline and as a result scored this criterion as -4. In assessing this criteria Cresswell *et al* states that information on the usage data in a spatial context was not available.

Specificity – The specificity criterion asks whether the consequence is both unmistakably defined and uniquely associated with the putative cause. It is concluded that dietary neonicotinoids, is certainly not uniquely associated with population decline in honey bees and score the specificity criterion -5.

Biological gradient – This criterion asks whether an increase in the power of the putative cause is reflected by an increased effect. Cresswell *et al* states that there currently is a lack of information on this issue, however he cites that in Europe, maize pollen can be a major component of the honey bee diet, and that a survey in Belgium found that the frequency of various depopulation symptoms, including colony mortality, in apiaries decreased as the neighbouring area of neonicotinoid-treated maize increased. On the basis of all the information available, it was concluded that the available evidence relating to biological gradient contraindicates dietary neonicotinoids as a cause of honey bee decline and as a result it was scored -4.

Strength – Cresswell *et al* concluded that 'the failure to detect a strong detrimental impact of trace dietary neonicotinoids under field conditions is a reasonable indication against their implication in honey bee declines' and as a result scored this criterion as -2.

Cresswell *et al* highlights that the proposition is 'reasonably justified cincture in the context of current knowledge' as it scored positively on all three of the theoretical criteria (i.e. 2, 3 and 4 above); however the proposition scored negatively on the associational criteria (i.e. 5, 6, 7, 8 and 9 above) and as a result Cresswell *et al* judged the circumstantial epidemiological evidence as substantially contraindicative. Overall, Cresswell *et al* concluded that 'virtually all of the circumstantial evidence clearly contraindicates the proposition'.

Cresswell *et al* highlights the issue that trace exposure is only one potential stressor and indicates that this could be an area of further work. In addition, Cresswell *et al* also highlights that further work could be carried out in the following areas: (i) experimental investigations; (ii) quantitative demographic model for honey bee population dynamics; (iii) epidemiological analysis of the association between the rates of neonicotinoid application and colony loss and finally, (iv) determine whether trace dietary neonicotinoids are synergists of co-acting stressors.

CRD views

The paper is clearly presented and is a useful addition to understanding of the issue in hand. Due to the nature of a published paper a full review of all the underlying data is not possible and this makes it a little difficult to fully understand how the conclusions and resulting scores were reached.

Of the areas of further work highlighted by Cresswell *et al* point (iii) is considered relatively straightforward, i.e. it should be possible to overlay information on usage along with information on colony losses to see if there is a clear pattern. This would use information from Bee health and PUSG. As for point (iv) it would be possible, albeit difficult, to carry out studies to determine if 'sick' bees were more susceptible to the effects of neonicotinoids compared to 'healthy bees' under field (or semi-field) conditions.

7. Honey bees (*Apis mellifera*) reared in brood combs containing high levels of pesticide residues exhibit increased susceptibility to *Nosema* (Microsporidia) infection.

Authors: Wu J.Y., Smart M.D., Anelli C.M., Sheppard W.S.

Published: Journal of Invertebrate Pathology 109 (2012) 326-329.

Summary

The paper examined the potential effects of developmental exposure to pesticide residues on subsequent susceptibility to *N. ceranae* infection in adult worker honey bees. Bees were raised from brood comb containing high or low residues of pesticide residues. There were two high residue combs. One high residue comb contained 10 pesticides – 2,4 dimethylphenyl formamide, chlorpyrifos, coumaphos, coumaphos oxon, endosulfan I, endosulfan II, esfenvalerate, fluvalinate, phosalone and THPI (a metabolite of captan); the other contained seven pesticides - 2,4 dimethylphenyl formamide, coumaphos, coumafos oxon, chlorothalonil, fluvalinate, permethrin total and pyrethrins. The control contained 'relatively low pesticide levels (coumaphos)'. Once raised the bees were then exposed to different levels of *N ceranae* spore inoculants. The study indicated that 'regardless of the colony environment (spores + syrup added or syrup only added), a higher proportion of bees reared from the high pesticide residue brood comb became infected with *N ceranae*, and at a younger age, compared to those reared in low residue brood combs'. The authors concluded that 'these data suggest that developmental exposure to pesticides in brood comb increases the susceptibility of bees to *N ceranae* infection.

CRD views

The paper is relatively brief and not particularly clear. As a result it is not possible to make many comments. The dosing regime is unclear. There is no information regarding the relevance of the concentrations given to the bees – i.e. were the levels appropriate and/or realistic? In addition the range of pesticides present is not that relevant to the UK as several are either no longer authorized or have never been authorised (it should also be noted that none of the pesticides are neonicotinoids).

Despite the above issues, the paper does raise the issue of exposure to a range of pesticides in the colony via the brood comb. This area is not currently considered as part

of the authorisation process. The risk assessment only looks at single products (although a single product may contain several active substances).

8. Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behaviour.

Authors: Mommaerts V., Reynders S., Boulet J., Besard L., Sterk and Smagghe G.

Published: Ecotoxicology (2010) 19:207-215 Doi 10.1007/s10646-009-0406-2

Summary

The paper outlines a bioassay to assess the impact of sub-lethal concentrations on the foraging behaviour of the bumblebee under laboratory conditions. The paper outlines a chronic toxicity assay that did not include foraging behaviour as well as one that did include foraging behaviour. As regards the former – adult bees were exposed orally to a range of concentrations of imidacloprid, thiamethoxam or thiacloprid. Observations of mortality as well as reproduction were made over an 11 week period. The endpoints from this first study were LC50 and EC50 as well as NOEC for survival as well as reproduction.

The second experiment consisted of two artificial nests (one with food the other with brood) connected with a tube of about 20 cm and use of queenless micro-colonies of 5 workers. Bees were trained to feed on untreated food and then this was replaced with treated food. Foraging behaviour was measured as well as mortality and reproduction. As for the first study, three neonicotinoids were assessed – imidacloprid, thiamethoxam and thiacloprid. The endpoints were LC50 and EC50 as well as NOEC for survival as well as reproduction.

Results from the first two experiments for imidacloprid are presented below

	Lethal effect (ppb)		Sub-lethal effect (ppb)	
	LC50	NOEC	EC50	NOEC
Imidacloprid				
Without foraging	59	10	3.7	20
With foraging	20	10	37	<2.5

The LC50 for thiamethoxam from the first study was 0.12 ppm, whilst that for thiacloprid was 18 ppm. The EC50 for thiamethoxam from the first study was 35 ppb, whilst that for thiacloprid was 12 ppm. NOEC were 10 ppb for thiamethoxam and 1.2 ppm for thiacloprid.

Endpoints from the second study for thiamethoxam and thiacloprid were not quoted in the paper.

Sub-lethal effects of imidacloprid on foraging behaviour in the greenhouse were assessed. Worker bees from colonies with queens were required to forage/fly for food that was placed at a distance of 3 m from their hives. Under the conditions of the study feed treated at the concentration of 2 ppb resulted in no effects.

The authors concluded that the experiments showed that concentrations that may be considered safe for bumblebees can have a negative influence on foraging behaviour. The study author also concluded that behaviour tests should be included in the risk assessment process.

CRD views

The study is a very detailed assessment of three neonicotinoids. It provides a detailed consideration of the design of two assays as well as highlighting that there needs to be a consideration of the effects at the semi-field scale. It is unclear as to the relevance of the concentrations tested and hence whether the results can be extrapolated to the field situation. It is however clear that this issue should be considered further.

SETAC considered the risk to non-apis bees and EFSA are also considering this issue.

The study recently commissioned by CRD (see above) should also help to indicate whether there is a concern under field conditions.

This study also shows the need to consider the appropriateness of trigger values when developing a risk assessment scheme and then to validate the scheme.

9. Assessment of the environmental exposure of honey bees to particulate matter containing neonicotinoid insecticides coming from corn coated seeds

Authors: Tapparo A., Marton D., Gioio C., Zanella A., Solda L., Marzaro M., Vivan L. and Girolami V.

Published: Environmental science and technology ACS dx.doi.org/10.1021/es2035152 Envion Sci Technol.

Summary

The paper has measured both the emitted particulate and the consequent direct contamination of single bees approaching the drilling machine during the foraging activity. The data indicate that the environmental releases of particles containing neonicotinoids can produce high exposure levels for bees, with lethal effects compatible with colony losses observed by beekeepers.

CRD views

This paper highlights that the dust from seed drills can pose a potential risk to honey bees. The amount of particulate matter emitted will depend upon the seed type as well the type of machinery being used. As a result of this, and other research mainly from Germany, CRD have started investigating issues related to the potential for particulate emissions from seed drills.

CRD has contacted the Agricultural Industries Confederation (AIC) to obtain a fuller picture on how neonicotinoid seed treatment in the UK is carried out. As a result of this we are confident that the set of circumstances that led to the German incident in 2008 are extremely unlikely to be reproduced here. This route of exposure will be considered as part of the new EU guidance document.

10. Ecological appropriate xenobiotics induce cytochrome P450s in *Apis mellifera*

Authors: Johnson R.M., Mao W., Pollock H.S., Guodong N., Schuler M.A., Bernbaum M.R.

Published: PLoS ONE 7(2): e31051. Doi:10.1371/journal.pone.0031051.

Summary

Honey bees rely, in part, on a suite of detoxication enzymes to metabolise naturally occurring xenobiotics and pesticides. The main enzyme is cytochrome P450 mono-oxygenase. Honey bees have fewer P450s than other insects and it has been proposed that this could be the reason why honey bees are sensitive to certain pesticides.

Amongst other findings the authors stated that non-honey diets significantly decreased the ability of honey bees to tolerate the natural toxin aflatoxin B1 yet had no measureable effect on toxicity of synthetic toxins tau-fluvalinate and imidacloprid. The LD50 of various pesticides was increased in the presence of a range of P450 inducers.

CRD views

This paper covered a range of biochemical issues – however the key points are that honey bees may not be able to process toxins as efficiently as other insects and that the toxicity may be affected by the diet are key.

Further consideration of these issues is necessary in understanding the potential risks. From a regulatory perspective it is perhaps more important to consider what the effects are rather than why they happen, hence the issue of detoxification and the relevance of diet should be considered under realistic field conditions.

11. RFID Tracking of Sub-lethal Effects of Two Neonicotinoid Insecticides on the Foraging Behavior of *Apis mellifera*

Authors: Schneider C.W., Tautz J., Grunewald B., Fuchs S.

Published: PLoS ONE 7(1): e30023. Doi:10.1371/journal.pone.0030023.

Summary

This study tested an experimental design using the radiofrequency identification (RFID) method to monitor the influence of sub-lethal doses of insecticides on individual honey bee foragers on an automated basis. With electronic readers positioned at the hive entrance and at an artificial food source, quantifiable data on honey bee foraging behaviour was obtained. Detailed information on flight parameters was also obtained. A comparison of several groups of bees, fed simultaneously with different dosages of a tested substance was carried out. With this experimental approach they monitored the acute effects of sub-lethal doses of the neonicotinoids imidacloprid (0.15–6 ng/bee) and clothianidin (0.05–2 ng/bee) under field-like circumstances.

The study authors state that residues of imidacloprid in an average nectar load from oilseed rape were 0.023-0.03 ng and when bees were exposed to doses of up to 3 ng no adverse effects were noted. As regards clothianidin all of the control and 0.05 ng/bee doses and 94.4% of the 0.5 ng/bees returned to the hive. 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.

CRD views

The paper provides information on a possible methodology to monitor honey bee activity at the colony level. This methodology is more efficient and easier to carry out compared to counting individual bees manually. The effects seen are of interest and indicate the potential effects, or rather lack of effects at appropriate concentrations.

This study is still artificial and hence the next logical step would be to employ this methodology at the field scale to see if the effects observed in this study were replicated under field conditions.

It is considered that this methodology should be considered in any future regulatory field trial carried out using honey bees.

12. The potential impacts of insecticides on the life history traits of bees and the consequences for pollination

Authors: Brittain C., and Potts S.G.

Published: Basic and applied ecology 12 (2011) 321-331

Summary

The paper considers the potential effects of both the lethal and sub-lethal impacts of insecticide use in agro-ecosystems on pollination services by bees. In particular the authors consider how particular life-history traits of pollinators, such as sociality and floral specialisation may be differentially affected by insecticides. The paper also considers the issue of pollination services as well as a trait-based approach.

CRD views

The paper is an interesting overview and contains much information which could help explain why effects are/are not being seen in the field. It is proposed that this paper and the issue of life-history traits should be considered when evaluating the risk assessment scheme being developed by EFSA.

13. Using video-tracking to assess sub-lethal effects of pesticides on honey bees (Apis mellifera L.)

Authors: Teeters B.S., Johnson R.M., Ellis M.D. and Siegfried B.D.

Published: (2012) Environmental Toxicology and Chemistry DOI 10.1002/etc.1830 – accepted preprint.

Summary

This study examined the utility of an automated video-tracking system, EthoVisionXT, to monitor the behavioural effects of sub-lethal exposure to tau-fluvalinate and imidacloprid. The following parameters were assessed: the distance that honey bees travelled in a 24-h period, the amount of time a pair of worker bees spent interacting, and the amount of time spent near a food source.

For each video-tracking experiment, 32 individual bees were randomly selected from a cohort of workers that had been anesthetized with carbon dioxide. Anesthetized bees were distributed into 16 polystyrene petri dishes (9 cm), two bees per dish. Each dish was bisected with a piece of 3-mm wire mesh to keep the pair separate but allow interaction. Each bee was provisioned with a 0.5 × 1.0-cm cube of sucrose agar for food and moisture. The 16 dishes were placed beneath a video surveillance camera on a frosted Plexiglas surface that was illuminated from below with an infrared light encased in a 45.72 × 53.34-cm plywood box. A total of 26 hours of bee activity was recorded, however the first and last hour was excluded to allow bees to recover from anesthetization and maintain consistent 24 hour tracks. A total of 32 arenas were defined with the software to establish where activity was to be tracked, and zones of interest were highlighted. Each petri dish consisted of two arenas, one for each bee, and the sucrose agar was identified as the "food zone." The software scanned each arena 15 times per second to determine the positions of all 32 bees simultaneously as time-series coordinates (x, y) within each arena. These coordinates were translated into actual distances by calibrating the program to the actual dimensions of the arena (9-cm diameter of the dish). A complete track record of the bees' movement patterns for the entire 24-h observation was obtained. The parameters investigated in this study were :

1. distance travelled (m) by each pair of bees – determined for each bee by summing up the distance between a bee's coordinates in consecutive samples
2. amount of time spent in the food zone (min) – the total time (total number of samples) that each bee was located on or adjacent to the sucrose agar cube
3. interaction time (min) between the bees that share a dish – defined as the number of samples in which the two bees in neighbouring arenas were located within 1.5 cm of each other, a distance at which bees were observed interacting through the screen divider.

Honey bee workers were treated topically with tau-fluvalinate or orally with imidacloprid. This was stated to replicate field condition. Imidacloprid was administered orally in sucrose agar containing 0.0, 0.05, 0.5, 5.0, 50, and 500 ppb imidacloprid, which was dissolved in distilled water and incorporated into the agar. The sub-lethal ranges were stated to correspond to LD10 and lethal concentration at 10% (LC10) estimates determined in preliminary bioassays. Bees were treated topically with 0.0, 3×10^{-4}, 1.5×10^{-3}, and 3×10^{-3} µg tau-fluvalinate in an acetone solution using a 50-µl syringe fitted to a repeating dispenser.

Analysis revealed that EthoVisionXT was capable of detecting differences in honey bee activity between treated and control groups for both tau-fluvalinate and imidacloprid. For distance travelled, bees treated with tau-fluvalinate moved significantly less than control bees at all dose levels. Bees exposed to 50 and 500 ppb imidacloprid also travelled significantly shorter distances. No statistically significant difference in distance travelled was observed between groups exposed to 0.05, 0.5, and 5.0 ppb imidacloprid. The effect of exposure on the amount of time treated bees spent in the food zone was affected by both tau-fluvalinate and imidacloprid. However, the Dunnett's test revealed that this was not significantly different from the control group for any dose level of tau-fluvalinate. Time in the food zone increased with higher levels of imidacloprid exposure. Although the group exposed to 0.05 ppb spent less time in the food zone than the control, each subsequent increase in exposure was accompanied by an increase in time spent near the sucrose.

The level of exposure also had a significant influence on the amount of time a pair of bees spent interacting when exposed to either tau-fluvalinate or imidacloprid.

CRD views

The primary aim of the study was to examine the ability of the video-tracking system to detect sub-lethal behavioural effects of tau-fluvalinate and imidacloprid on worker honey bees. The study illustrated that it is capable of measuring these movements. The authors indicate that this measuring approach could be used as part of a regulatory study, i.e. it could be used to measure the effects of pesticides on honey bees in the laboratory. As regards the effects seen, it is important to note that they need to be related to what is likely to be encountered either in the hive (i.e. were the rates used realistic in terms of exposure to tau-fluvalinate) or in the field (i.e. were the rates used realistic in terms of what a worker honey bee is likely to encounter in the field.)

14. Parasite-insecticide interactions: a case study of *Nosema ceranae* and fipronil synergy on honey bee.

Authors: Aufauvre J., Biron D.G., Vidau C., Fontbonne R., Roudel M., Diogon M., Vigues B., Belzunces L.P., Delbac F., & Blot N

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Summary

The aim of this study was to determine potential synergistic interactions in honey bees. *Nosema ceranae* and a sub-lethal dose of the insecticide fipronil were chosen as natural and chemical stressors respectively. The key issue with this work was the sequence of exposure and whether this resulted in different reactions. With this in mind, the following treatments were assessed:

(a) both treatments were applied on emerging honeybees,

(b) bees were chronically exposed to fipronil from week 1 (i.e. from emergence) then infected with *N. ceranae*,

(c) bees were infected with *N. ceranae* at their emergence then chronically exposed to fipronil for week 2,

(d) both treatments were applied on 7-day-old bees

A concentration of 1 µg/L fipronil in sucrose-syrup was used. It was stated that this concentration was based on the concentration that could be encountered in a hive.

Survival analysis indicated that each *N. ceranae*-fipronil combination led to a significant decrease in honey bee survival compared to either the control or single treatments. Control honey bees had the lowest mortality with 24% at the end of experiment, 22 days

after emergence. Mortality when honey bees were exposed to either *N. ceranae* or fipronil alone reached a maximum of 39 and 31%. The effect on fipronil was not statistically significant. When exposed to both the cumulative mortality results were as follows:

(a) both treatments were applied on emerging honeybees = 83.7%

(b) bees were chronically exposed to fipronil from week 1 (i.e. from emergence) then infected with *N. ceranae* = 66.5%

(c) bees were infected with *N. ceranae* at their emergence then chronically exposed to fipronil for week 2 = 81.4%

(d) both treatments were applied on 7-day-old bees = 71.9%

In each case, the *N. ceranae*-fipronil combination induced a synergistic effect compared to the sum of the effects observed in honeybees exposed to each stressor alone.

Statistical analysis indicated that *N. ceranae* factor had a highly significant impact on honey bee survival, but only when applied at emergence. Fipronil also had a highly significant impact on honeybees' survival probability when applied at their emergence and a less significant impact when applied on 7-day-old bees. Moreover, the factor corresponding to the sequence of treatments also had a highly significant impact on survival.

On the basis of food consumption it was determined that honey bees absorbed a daily mean quantity of fipronil of 1/254th of the LD50 (stated to be equivalent to 16.4±1.6 pg/day/bee) during week 1 and 1/179th of the LD50 (stated to be equivalent to 23.3±2.5 pg/day/bee). The LD50 used to determine these exposure estimates was 4.17 ng/bee (this is the same as the agreed Annex I endpoint for the acute oral LD50 for honey bees). Infected honey bees did not significantly consume different cumulated quantities of fipronil compared to uninfected honey bees.

The number of spores present in the abdomen of surviving honey bees at the end of the experiment was determined. Low levels were detected in the control honey bees (i.e. $3.0x10^3 \pm 10.3x10^3$ spores/bee). Spore counts were higher at the end of the study in honey bees exposed at emergence compared to honey bees exposed on day 7 onwards. It was assumed by the study authors that this was due to the study duration.

To identify a potential impact of the fipronil exposure on spore production, spore counts were compared between honey bees that were infected on a same day. The text states that among groups infected at emergence, honey bees only infected with N ceranae had a lower spore count compared to honey bees intoxicated during week 2. This is not in line with figure presented. Similarly, there is a statement regarding antagonistic effects, however this was not apparent from the data presented.

CRD views

Fipronil is no longer authorised in the UK hence the direct relevance is limited. However, the general issue of synergism is important and this study indicates that the order of exposure may be important. One interesting result is that in uninfected bees, fipronil wasn't having an effect on survival. There is a lack of information regarding the relevance of the level of infection. Meana (2010) has reviewed the use of spore counts and states that spore count has been rejected as a marker of health status in naturally infected colonies.

Fera has also looked at the paper and has pointed out a potential anomaly/error in Figure 1 in that the level of mortality in the control appears to be different, when it should be the same. In addition, Fera has highlighted that *Nosema* may be damaging the midgut which is important in bees for detoxication. The issue of exposure to pesticides and disease levels is being investigated in the Research and Development project (PS 2370).

15. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment

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Summary

This work was supported by the Ministry of Economic affairs, Agriculture and Innovation of the Netherlands (Project BO-12.01-001-003-PRI-1) and the Fund for Scientific Research (FWO)-Flanders (Belgium).

The focus of the paper was on three different key aspects determining the risks of neonicotinoid field concentrations for bee populations:

1. the environmental neonicotinoid residue levels in plants, bees and bee products in relation to pesticide application,
2. the reported side-effects with special attention for sub-lethal effects, and
3. the usefulness for the evaluation of neonicotinoids of an already existing risk assessment scheme for systemic compounds.

The paper is very detailed and includes several summary tables. It is not proposed to repeat these here. It reviewed all the publicly available data and is a comprehensive review and included several papers already considered in the review of the Buglife report, as well as more recent publications. The study authors concluded the following:

Many lethal and sub-lethal effects of neonicotinoid insecticides on bees have been described in laboratory studies, however, no effects were observed in field studies with field-realistic dosages.

The results obtained so far for neonicotinoids (mainly for imidacloprid) under laboratory conditions do not give a good estimation of the real effect on honey bees under field conditions.

In addition, the authors have highlighted the following:

- There should be some consideration of guttation
- Difficulty in extrapolating from laboratory studies to potential effects under field conditions.
- The few reported residue levels of neonicotinoids in nectar (average of 2 µg/kg) and pollen (average of 3 µg/kg) were below the acute and chronic toxicity levels; however, there is a lack of reliable data as analyses are performed near the detection limit.
- The risk assessment scheme for soil-applied systemic pesticides proposed by EPPO seems adequate for assessing the risks of side effects by neonicotinoids as it takes into account the effect on different stages (adult versus larvae) and on different levels of biological organization (organism versus colony). Nevertheless, there is still a need for testing field-realistic concentrations at relevant exposure and durations and, especially for honey bees, to continue side-effect evaluation over winter and the next year in spring.
- On the basis of two studies, the authors have concluded that these studies demonstrated no long-term effects on honeybee colonies of environmentally relevant concentrations

CRD views

This is a comprehensive review, however it would have benefited from greater clarity regarding some of the studies considered, for example how were the LC50 data produced. In addition, the section on the risk assessment would have benefited from actually using some of the data as well as greater clarity regarding which scheme they were referring to. As regards the overall conclusion, these are in line with our own assessment, except to highlight that the main conclusion regarding effects observed in the field is based on a relatively small dataset.

Annex 2: The regulation of pesticides

Agricultural and home and garden use pesticides, known as plant protection products (PPPs) are regulated under EU rules. Active substances are approved at EU level. If an active substance meets EU safety requirements, products containing that active substance can be authorised at Member State level. This authorisation is carried out according to common rules, but takes into account national agronomic, climatic and dietary circumstances.

Authorisation or approval is only granted if assessment of scientific data shows that risks are acceptably low. Benefits of the pesticide are not taken into account. The risk assessment addresses risks to honey bees (the process is outlined in Annex 3) and to two other non-target arthropods but not, specifically, to other bee species.

Pesticide manufacturers submit a Dossier containing all the required information - including study methodology and data generated together with their conclusions. Studies must be conducted to internationally recognised guidelines where these are available, and have verified Good Laboratory Practice quality assurance compliance. The Dossier is scrutinised and assessed by experts from a national regulatory authority in all of the various scientific disciplines involved. The regulatory authority's opinion - which may or may not coincide with the company's - then appears in the Draft Assessment Report (DAR) of the substance in question.

The DAR produced by the regulatory authority is submitted to the independent European Food Safety Authority (EFSA) who organise a peer review by experts from Member States. Following this peer review, EFSA produce a conclusion which is sent to the Commission for final scrutiny by the Member States and for adoption through a committee procedure. The DARs and EFSA conclusions are published on the EFSA website (http://www.efsa.europa.eu). Active substance authorisations are normally for ten years and are then subject to complete reassessment according to current standards. Both the EU and individual Member States are able to carry out an earlier reassessment if new information of concerns comes to light.

In the UK, Defra has lead responsibility for plant protection products. The regulatory system is run, under our direction, by CRD. Pesticides can only be sold or used if they are approved and conditions are routinely attached to approval (for example specifying dose rates, timing and place of application) to ensure protection of human health and the environment (including wildlife).

A Commission Directive (2010/21/EU) sets specific provisions relating to seed treatment use of clothianidin, imidacloprid, thiamethoxam and a non-neonicotinoid pesticide called fipronil. These provisions relate to labelling of pesticide-treated seed, a requirement for professional application of seed treatments to seed, and monitoring for possible impacts on bees. The Directive does not apply to acetamiprid and thiacloprid, which are little used as seed treatments and show acute toxicity to bees several orders of magnitude less than the other three neonicotinoids (acetamiprid and thiacloprid are cyano-substituted neonicotinoids while the others are nitroguanidine-substituted).

Annex 3: Honey bee risk assessment under EU pesticide regulations

For pesticides that are applied as a *spray*

Data on the acute oral and contact toxicity of the pesticide is always submitted when there is likely to be exposure to foraging honey bees. Exposure could result from honey bees foraging the crop that is being sprayed or from the honey bees foraging weeds in the crop.

These data are generated via the use of internationally agreed test guidelines[1]. The endpoints from these studies are LD50, i.e. the median lethal dose that results in 50% mortality of the test population. Two separate studies are conducted: acute contact toxicity is determined by placing a dose of the pesticide on to the thorax of the bee; – acute oral toxicity is determined by feeding bees treated sucrose. These are laboratory based studies that are carried out under controlled conditions and use either the active substance or the formulated pesticide product.

The LD50 is then used to derive a 'hazard quotient' – the application rate of the pesticide in g/ha divided by the LD50 in µg/bee. If the resulting ratio is less than a trigger value of 50[2], it is considered that an unacceptable level of mortalities are unlikely to occur and the pesticide can be authorised without any restrictions regarding the risk to honey bees. If the ratio is greater than 50 then the product is either restricted to a time when honey bees are not foraging or further data are requested to enable a decision to be made on authorisation.

If a restriction is imposed, the UK product label will carry the following wording:

> *Dangerous to bees. To protect bees and pollinating insects do not apply to crop plants when in flower. Do not use where bees are actively foraging. Do not apply when flowering weeds are present.*

If further data are requested, these take the form of either semi-field studies (sometimes referred to as cage studies) or field studies. Semi-field studies use a small colony of about 5,000 bees, which is placed inside the enclosure a few days before the crop is sprayed. The crop is sprayed once the bees have become accustomed to the enclosure and are actively foraging the crop. The following endpoints are considered – mortality, foraging activity and survival of the colony. Semi-field studies usually last only a few days. There is always a control enclosure and there should be sufficient replication to permit statistical analysis.

Field studies are large scale and involve an unenclosed crop where honey bee colonies are placed adjacent to the crop. If a study was being conducted on oilseed rape then a

[1] See Organisation for Economic Cooperation and Development guideline for the testing of Chemicals – honey bees, acute oral toxicity test (OECD 213) and acute contact test (OECD 214).

[2] This value of 50 has been validated see Aldridge, C. A., and A.D.M. Hart. 1993. Validation of the EPPO/CoE risk assessment scheme for honeybees, Appendix 5. Proceedings of the 5th International Symposium on the Hazard of Pesticides to Bees, 26Ð28 October 1993, Plant Protection Service, Wageningen, The Netherlands.

plot of approximately 1 ha would be used. Colonies are used that contain at least 10,000 bees and each colony should cover at least 10–12 frames, including at least 5–6 brood frames. The crop is sprayed once the bees have become accustomed to the crop and are actively foraging. The major effects that are monitored as part of a field study are effects on mortality, foraging activity and survival of the colony. Further details regarding how these studies are carried out is provided in internationally developed guidance[3].

The effects observed in the semi-field or field study will determine whether the pesticide is authorised and whether restrictions are applied.

For pesticides that are applied as *seed treatments* or as a *solid* formulation

Some pesticides are applied directly to seed prior to drilling in order to protect them from soil pests and soil borne diseases. If the pesticide is systemic (i.e. it can move into the plant and hence occur in the flower) then honey bees may be exposed to it. If this is considered likely, then a risk assessment is carried out. The above 'hazard quotient' approach is not appropriate for assessing this risk and so reliance is currently placed on semi-field and field studies, similar design in design to those outlined above. A similar approach is use for pesticides formulated as granules or pellets. The effects observed in the semi-field or field study will determine whether the pesticide is authorised and whether restrictions are applied.

Development of the risk assessment

The risk assessment continues to be developed. Applicant's will in future need to submit additional data covering: effects on honey bee brood development and other honey bee life stages (this information will enable an assessment of any effects on the development of the brood that may result); and potential chronic effects on adult bees.

The European Food Safety Authority (EFSA) Panel on Plant Protection Products and their Residues (PPR) published on 23 May a review of the science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp and solitary bees). Following publication of this opinion, EFSA, the European Commission and Member States will develop guidance that will be used as a part of the authorisation process with Europe. UK experts are actively involved in this work.

[3] See European and Mediterranean Plant Protection Organisation (EPPO) Side effects on honey bees PP 1/170(4).

Annex 4: ACP advice: neonicotinoids and bees

Overall, the ACP were agreed that the current risk assessments are secure and have concluded that there is no justification to take regulatory action at present. Furthermore, there is no evidence as yet of neonicotinoid impacts on bees in the UK. However, the ACP will consider any new information as it arises and keep the situation under close review. An explanation of the work leading to this advice is set out below.

1. The ACP has examined in detail the recent publications in the scientific literature. They identified a number of points at a first discussion of this topic at the May 2012 meeting which have now been followed up.

2. Members have carefully reconsidered the data (including an examination of the raw data) supporting the current authorisations for thiomethoxam products in the light of findings from recent published data (specifically the paper by Henry et al) and EFSA discussions. The field studies submitted by the applicants are fully compliant with current regulatory guidance and additionally cover some aspects not required by the current guidance (e.g. over-wintering). In line with current guidance the regulatory studies were not designed with detailed statistical analysis in mind, and their power to detect statistically significant changes is not established. Also, they would not show some of the specific sub-lethal effects suggested by academic studies, such as disorientation over distances. However hives exposed to treated crops did not show any gross effects on a wide range of important endpoints when compared to control hives exposed to untreated crops.

3. While noting there were some questions concerning aspects of the two published studies (by Henry et al and Whitehorn et al), the ACP cannot discount their findings. The Committee believe these studies provide interesting information that should be considered in the development of future regulatory guidance. Some further research is merited in the light of these papers and others to clarify the findings and their relevance to the UK field situation. The ACP is pleased to note that relevant work is already underway.

4. This further work will need time to be completed. In particular the ACP is aware that the study on bumble bees (Defra project PS 2371) is currently in its field phase and it is expected results will be reported in March 2013. The ACP has asked for preliminary information to be made available as soon as possible following the field phase this autumn/winter. The study examining residues in honey bees (Defra project PS2370) to assist in the interpretation of the relationship between pesticides residues and disease in bees is also expected to report in March 2013. A preliminary examination of bee health statistics following the introduction of the neonicotinoids is expected to become available later this summer. Finally the EFSA work re-evaluating all of the neonicotinoid insecticides in the light of the latest research and the development of the revised guidance on assessing risk to bees are both due by the end of this year. The ACP will keep this work and its potential impact on authorisations under review

5. The ACP also identified a number of other possible areas for research into the possible impacts of neonicotinoid insecticides. These include some work on bee toxicokinetics to examine factors related to dose and exposure period, a true field study looking at disorientation (while recognising the very real practical difficulties might make this impossible to do). The ACP also asked their Environmental Panel to look at work on guttation as a potential source of exposure to other non-target arthropods.

6. Although the ACP has considered thiamethoxam in detail, the Committee agreed that the conclusions reached can be applied broadly to the authorisations of other neonicotinoid insecticides because:

    - The acute toxicity of thiamethoxam, clothianidin and imidacloprid are all of a similar order of magnitude, with similar extent of use. Acetamiprid and thiacloprid are significantly less acutely toxic and are used on a significantly smaller area.
    - The chemical properties of all of the neonicotinoid insecticides are very similar and the mode of insecticidal action is identical for them all.

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