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Existing Scientific Evidence of the Effects of Neonicotinoid Pesticides on Bees

NOTE

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Abstract

Reports about bee colony losses and damage have increased in recent years all over Europe. Neonicotinoids, a class of systemic insecticides, are more frequently associated with the pollinator declines. The present briefing note gives an overview about neonicotinoid uses and recent scientific findings on their impact on bee colony survival and development. Risk-mitigation measures aimed at protecting non-target organisms (such as bees), are outlined and discussed.

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LIST OF ABBREVIATIONS

ANSES	French Agency for food, environmental and occupational health safety
DEFRA	Department for Environment, Food and Rural Affairs, UK
EC	European Commission
EU	European Union
EFSA	European Food Safety Authority
LD_{50}	The dose required to kill half of a test population after a specified test duration
RFID	Radio Frequency Identification Methodology

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EXECUTIVE SUMMARY

KEY FINDINGS

- Although bee declines can be attributed to multifarious causes, the use of neonicotinoids is increasingly held responsible for recent honeybee losses.
- Neonicotinoids show high acute toxicity to honeybees.
- Chronical exposure of honeybees to sub-lethal doses of neonicotinoids can also result in serious effects, which include a wide range of behavioural disturbances in bees, such as problems with flying and navigation, impaired memory and learning, reduced foraging ability, as well as reduction in breeding success and disease resistance.
- Recent scientific findings are urging to reassess the bee safety of approved uses of neonicotinoid insecticides at European level. A current review, carried out by the European Food Safety Authority EFSA (on behalf of the European Commission) will give new insights into this issue.
- As long as there are uncertainties concerning the effects of neonicotinoids on honey bees, the precautionary principle in accordance with the Regulation (EC) No 1107/2009 should be applied when using neonicotinoids.

Pollination, provided by a great variety of bees and other insects, represents a vital ecosystem service. For Europe it is estimated that more than 80% of all crops rely at least to some extent on insect pollination. Against this background, the increasing number of reports about colony losses and damage inflicted on honeybees and other wild pollinator species throughout Europe is of great concern. For the most part, declines are attributed to an interaction of various factors. However, pesticide use is more and more under the suspicion of having a significant impact on bee mortality. Particularly neonicotinoids, a widely used group of systemic insecticides, are held responsible for recent bee declines. Besides the common ways of exposure, their systemic character enables them to migrate through the entire plant all the way to the flowers, potentially causing chronic low dose exposure to pollinators.

Besides giving the acute toxicity profiles of neonicotinoids, this briefing note gives an overview about the findings of recent studies on the sub-lethal effects of these systemic pesticides. Reported sub-lethal impacts on honeybees include various behavioural disturbances, such as reduced homing ability, impaired memory and learning, as well as negative impacts on the ability of worker bees to forage and communicate. Other studies found that the chronic exposure to low doses of neonicotinoids can reduce the breeding success of bees and lead to a neonicotinoid-induced reduction in disease resistance. Thus, a widespread conclusion of different authors is that neonicotinoids can contribute to lethality even at low doses by making bee colonies more vulnerable to other disruptive factors. Although existing research documents measure the sub-lethal effects, the results are sometimes put into question. Recent scientific findings are urging for an update of the risk assessment of all neonicotinoid insecticides approved at European level and their effects on bees. In order to fully assess the risk to bees, it is necessary to carry out additional, and properly designed, field studies which are conducted over a long period of time. So far various European countries have implemented measures which aim to avoid possible negative effects of neonicotinoid applications on bees.

However, risk mitigation measures on EU and national level concentrate on reducing the risks from acute poisoning of bees, but they do not consider the risks of chronic exposure to sub-lethal doses. New insights can be expected from a current review performed by the European Food Safety Authority EFSA on behalf of the European Commission. Further action on EU-wide level is not expected before this new assessment is available.

Several recent publications suggest that exposure to different classes of neonicotinoids even at very low doses reduces the fitness of bees. As long as these and other questions remain unclear the precautionary principle in accordance with Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market should be applied, ensuring a high level of protection of both human and animal health and the environment.

1. INTRODUCTION

Bees, including honeybees, bumble bees and solitary bees play an important role in the creation and conservation of biodiversity and are an important economic factor in providing essential pollination for a wide range of crops and wild plants. Insect pollination describes not only an ecosystem service, but also a production practice of which farmers are making full use for crop production (cf. GALLAI et al. 2009). According to BLACQUIÈRE et al. (2012) bees represent the most prominent and economically most important group of pollinators worldwide. About 35% of the world food crop production, which accounts for an annual value of 153 billion Euros (cf. GALLAI et al. 2009), depends on pollinators (cf. KLEIN et al. 2007; BLACQUIÈRE et al. 2012). In Europe it is estimated that 84% of all cultivated agricultural crop species and 80% of all wild plants are at least to some extent depending on insect pollination (BLACQUIÈRE et al. 2012).

According to the Communication on Honeybee Health from the European Commission (COM (2010)714final), the number of beekeepers in the EU is estimated to be about 700,000 accounting for about 15 million hives (ALIX et al. 2011). During the last few years, the number of reports from different European countries about honeybee colony losses or damage has increased (cf. POTTS et al. 2010; NEUMANN & CARRECK 2010; TOPOLSKA 2008; GIRSCH & MOOSBECKHOFER 2012). Furthermore, there is also growing evidence of a decline in European wild pollinator species (POTTS et al. 2010; POTTS et al. 2011; UNEP 2010). Populations of honeybees and other pollinators have declined worldwide in recent years, so that there is global concern especially about a phenomenon referred to as "Colony Collapse Disorder", which is characterised by the rapid loss from a colony of its adult worker bee population, as currently observed in the USA (HOPWOOD et al. 2012).

Given the importance of honeybees (and wild species) for pollination and human nutrition, a considerable amount of research on the decline in pollinator insect populations has been carried out over the last few years. While there is a consensus that the ectoparasitic mite *Varroa destructor* was a major contributor to bee mortality in former periods (following its arrival in Europe in the 1970s), opinions differ as to what the drivers of more recent losses are (STOKSTAD 2007; POTTS 2010). Current declines in pollinators are frequently attributed to interactions of various factors, such as colony management, habitat losses, honeybee pests and parasites, as well as environmental and anthropogenic elements (cf. HOPWOOD et al. 2012; MAINI et al. 2010; GIRSCH & MOOSBECKHOFER et al. 2012). Research is being directed at identifying those individual stressors which are most strongly associated with pollinator decline. Pesticide use is one of the factors under consideration. In spite of the fact that honeybees are generally exposed to a wide range of pesticides (cf. MULLIN et al. 2010), neonicotinoids - a class of systemic insecticides - are more and more under the suspicion of making a significant contribution to bee mortality (cf. SETAC 2011).Several beekeepers and scientists in Europe and all over the world hold the widespread use of insecticides from the neonicotinoid group responsible for current honeybee colony losses and damage.

Neonicotinoids were introduced in the early 1990s and today they are among the most widely used crop pesticides worldwide. Within the last few years, various member states of the European Union have taken regulatory action to restrict the use of specific neonicotinoids, with the intention to protect the honeybees. The fact that there is a wide range of different national approaches to the approval of these systemic insecticides indicates that the regulatory authorities have different interpretations of current scientific evidence. These differences also reflect

- existing uncertainties about the effects of low-dose exposure on bee health and
- different stakeholder views, the concerns of beekeepers as well as different agricultural practices within EU member states (Pesticide Action Network UK 2012).

Currently, the European Food Safety Authority (EFSA) is carrying out a review of neonicotinoids and their impacts on bee health: The European Commission has instructed the EFSA to review (according to Article 21 of Regulation (EC) No 1107/2009)[1] the current bee risk assessment for all neonicotinoid insecticides approved at European level[2] as well as for all authorised uses. The work is scheduled to be completed by the end of 2012. As noted by the Standing Committee on the Food Chain and Animal Health in July 2012, the majority of the member states intend to wait for the results of this risk assessment of bee exposure to such substances before any decisions about EU-wide measures are taken.

[1] Regulation 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, OJ 2009 L 309.

[2] Thiametoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid

2. MODES OF ACTION

The mode of action of neonicotinoid pesticides is similar to nicotine, a natural plant compound which once was widely used as an insecticide. Neonicotinoids are agonists of nicotinic acetylcholine receptors which are normally activated by the neurotransmitter acetylcholine (cf. LIU et al. 2006). Thus, neonicotinoids block an intrinsic chemical pathway which transmits nerve impulses to the insect's central nervous system. This causes excitation of the nerves and can result in paralysis and death. As neonicotinoids block a specific neuron pathway, which is more abundant in insects than in mammals or birds, these insecticides are selectively more toxic to insects than to other classes of animals (HOPWOOD et al. 2012; TOMIZAWA & MOTOHIRO 2004).

Neonicotinoids are also systemic, which means that they are absorbed and transported into all parts of the plant tissue and in this manner offer protection against sucking or chewing insect pests. Plants can take up the chemicals through their roots or leaves, while subsequently the vascular tissues incorporate the chemical into the stems, flowers (also the nectar and pollen), leaves or even the fruits (HOPWOOD et al. 2012).

According to the Dutch toxicologist Dr. Tennekes, neonicotinoid insecticides cause irreversible and cumulative damage to the central nervous system of insects. The Druckrey-Küpfmüller equation states that if both receptor binding and the effect are irreversible, exposure time would reinforce the hazardous effect (TENNEKES 2010a).

3. WAYS OF EXPOSURE

Neonicotinoids can be applied as foliar sprays, seed coatings, soil drenches or granules, as well as by direct injection into tree trunks or by chemigation (additive to irrigation water). Due to this wide variety of application methods, and in combination with their systemic properties and their low toxicity to vertebrate species, neonicotinoides are increasingly used for crop protection against insect pests in Europe and all over the world (HOPWOOD et al. 2012, STOKSTAD 2012). Thus, honeybees are frequently exposed to these systemic substances. Within the European Union approximately 70% of the neonicotinoids used on fields are applied by spraying, whereas less than 20% of the applications are seed treatments and another nearly 20% are methods such as drip irrigation, soil disinfectants, etc. (EFSA 2012a). While successfully controlling a variety of agricultural crop pests, these applications do not only affect insect pests but also non-target organisms like pollinator species (BLACQUIÈRE 2012).
Systemic pesticides such as neonicotinoids are absorbed into plant tissues. There are thus additional exposure routes besides the common ways of exposure to sprayed pesticides (cf. HOPWOOD et al. 2012). Neonicotinoids are able to migrate through the entire plant all the way to the flowers, which potentially causes toxic chronic exposure to non-target species like pollinators (UNEP 2010). KRUPKE et al. (2012) found in their studies that residues of neonicotinoids tend to be incorporated into weeds growing within or next to treated fields, which indicates either a deposition of neonicotinoids on the flowers, or an uptake by the root system, or both.

These systemic chemicals have longer durations of action than other pesticides. According to HOPWOOD et al. (2012) they are able to remain in plant tissues for months or even for more than a year. In addition to that, neonicotinoids are able to remain in soils over longer periods of time (see Table 1). The soil half-life of clothiadinin or imidacloprid for instance varies between a few months and two or three years, depending on the soil type. Untreated plants are at risk of residue uptake from previous uses of pesticides still remaining in the soil (cf. HOPWOOD et al. 2012). As neonicotinoids are soluble in water there is also a risk of migration to surface water bodies (cf. VAN DIJK 2010). Besides leaching, water sources may also be contaminated by oversprays, drifts or field run-offs. On warm days bees are generally gathering water to cool their hives – contaminated water bodies are thus additional routes of exposure (HOPWOOD et al. 2012).

Table 1: The half-life of neonicotinoids in soils

Neonicotinoid	Half-life in soil (aerobic soil metabolism)
Acetamiprid	**1-8 days**
Clothianidin[3]	**148-1,155 days**
Imidacloprid	**40-997 days**
Thiacloprid	**1-27 days**
Thiamethoxam	**25-100 days**

Source: HOPWOOD et al. 2012

[3] Clothianidin is a primary metabolite of thiamethoxam

Bees and insects depending on nectar, pollen or other floral resources are increasingly exposed to the residues of neonicotinoids or their metabolites, when they feed on treated plants (cf. HENRY et al. 2012, WHITEHORN et al. 2012, KRISCHIK et al. 2007). As neonicotinoids are absorbed by the plant and transferred through the vascular system, the plant becomes toxic to sucking or chewing insects (cf. HOPWOOD et al. 2012). Residue levels are thus also found in the nectar or pollen of treated plants. Typical levels are usually not lethal, but there is growing evidence from laboratory tests that chronic exposure to low doses of these systemic insecticides are also harming the bee populations (cf. STOKSTAD 2012).

Residues of neonicotinoids could also be evidenced within other plant exudates such as guttation water[4]. GIROLAMI et al. (2009) for instance investigated leaf guttation drops of corn plants germinated from neonicotinoid-coated seeds. It was found that the concentration of neonicotinoids in guttation drops can be near those of active ingredients commonly applied in field sprays for pest control, or even higher. The resarchers around GIROLAMI discovered that bees who consumed guttation drops, collected from plants grown from neonicotinoid-coated seeds, encountered death within a few minutes.

Another route of exposure to neonicotinoid residues is the dispersal of contaminated dust from dressed seeds during sowing (GREATTI et al. 2003, KRUPKE et al. 2012). Solid coating debris and uplifting from sowing machines can potentially fall over nearby wildflowers and lead to contamination (cf. GIROLAMI et al. 2009). In spring 2008 declines of bee colonies were observed in Germany (Rhine valley), Italy and Slovenia during and after sowing of clothianidin-coated maize seed with pneumatic seed drills. Similar analyses in Germany verified the causal connection between the use of this seed dressing insecticide and the reported damage in honeybee colonies (HEUVEL 2008). For Austria GIRSCH & MOOSBECKHOFER (2012) found correlations between the occurrence of honeybee losses in maize and oilseed rape production areas treated with neonicotinoids from 2009 to 2011.

[4] Guttation represents a natural plant phenomenon causing the excretion of xylem fluid at leaf margins (cf. GIROLAMI et al. 2009)

4. AUTHORISATION OF NEONICOTINOIDS

According to Council Directive 91/414/EEC, which was in force when neonicotinoid pesticides were first authorised, pesticides can only be approved at EU level if their use does not cause unacceptable effects on the environment (e.g. on bee health) (EUROPEAN COMMISSION FACTSHEET 2009). So, a risk assessment was established for each active ingredient by a rapporteur member state and discussed with all members of the European Union.

The current risk assessment for honeybees relies on a Hazard Quotient (HQ) approach (application rate/LD_{50}) in lower tiers and on semi-field and field tests in higher tiers. The final decision on protection goals needs to be taken by risk managers. There is a trade-off between plant protection and the protection of bees: The effects on pollinators need to be weighed against increases in crop yields achieved through better protection of crops against pests. An overview of available studies on sub-lethal doses and long-term effects of pesticides on bees highlights gaps of knowledge and research needs in the following areas: More toxicological studies need to be performed on bees for a wider range of pesticides and their effects on both adults and larvae including sub-lethal endpoints, and also including contact and inhalation routes of exposure (EFSA 2012b).

Certain neonicotinoids which are used as plant protection products have been authorised. Annex I to the Commission Implementing Regulation (EU) 540/2011 lists acetamiprid (No 91), clothianidin (No 121), thiamethoxam (No 140), thiacloprid (92) and imidacloprid (No 216).

Now Council Directive 91/414/EEC has been replaced by the new European Parliament and Council REGULATION 1107/2009, with the same goal to protect non-target species by laying down rules for the approval of active substances used in plant protection products. Article 21 of this regulation provides for a review process of authorised substances in case new scientific and technical evidence indicates that the substance does not fulfil the approval criteria set out in the regulation. The Commission is taking measures to avoid accidents and has reinforced the conditions for placing on the market and for using insecticides that are mostly employed as seed treatments and, in particular, thiametoxam, clothianidin, imidacloprid and fipronil (COMMISSION DIRECTIVE 2010/21/EU). The Commission has also launched a whole review of the risk assessment of all neonicotinoids and the risks they pose to bees, namely of thiametoxam, clothianidin, acetamiprid, thiacloprid and imidacloprid, which is expected to be finalised by 31 December 2012.

5. TOXICITY

Neonicotinoids show high acute toxicity to honeybees. The acute oral and the acute contact toxicity are in the range of nanogram (ng) to microgram (µg). For details see the LD_{50} values listed in Table 2.

LD_{50} is the dose required to kill half of a test population after a specified test duration. The general rule is: the lower the LD_{50} value, the higher the toxicity level of a substance. LD_{50} figures are frequently used as a general indicator of a substance's acute toxicity.

For acute oral toxicity, adult worker honeybees are fed with the test substance dispersed in sucrose solution. For acute contact toxicity, adult worker honeybees are exposed to the test substance dissolved in an appropriate carrier which is directly applied to the thorax.

Table 2: Acute toxicity to bees

Substance	Acute oral toxicity	Acute contact toxicity
Acetamiprid	LD_{50}: 14.53 µg/bee	LD_{50}: 8.01 µg/bee
Clothianidin	LD_{50}: 0.00379 µg/bee	LD_{50}: 0.04426 µg/bee
Imidacloprid	LD_{50}: 0.0037 µg/bee	LD_{50}: 0.081 µg/bee
Thiacloprid	LD_{50}: 17.32 µg/bee	LD_{50}: 38.82 µg/bee
Thiamethoxam	LD_{50}: 0.005 µg µg/bee	LD_{50}: 0.024 µg/bee

Source: List of endpoints in review reports and in the Draft Assessment Reports

Furthermore, various forms of semi-field testing (cage, tunnel or tent tests) as well as field testing were conducted to show possible effects under more realistic conditions.

Generally, oral toxicity appears to be higher than contact toxicity (one order of magnitude). Thiacloprid and acetamiprid are cyano-substituted neonicotinoids while clothianidin, imidacloprid and thiamethoxam are nitroguanidine-substituted neonicotinoids. There are data to suggest that the former are readily metabolised in bees and that they have considerably lower acute toxicity profiles for bees than the nitroguanidine-substituted neonicotinoids. Imidacloprid, thiamethoxam and clothianidin show a similar acute toxicity profile, while thiacloprid and acetamiprid are less toxic (10000-fold). Therefore, the focus should be on imidacloprid, thiamethoxam and clothianidin.

6. SUB-LETHAL EFFECTS OF NEONICOTINOIDS

Next to being acutely toxic in high doses, exposure to neonicotinoids can also result in serious sub-lethal effects if insects are chronically exposed to low doses. Sub-lethal effects are considered as impacts on the physiology and behaviour of an individual that has been exposed to a pesticide without directly causing death (cf. SCHNEIDER et al. 2012). For a complete analysis of the impact of neonicotinoids, both direct mortality and sub-lethal effects have to be considered.

According to the NGO PAN Europe, sub-lethal toxicity to bees and other pollinators is the most likely exposure scenario in the field from neonicotinoid seed treatments. This is due to the fact that the concentrations detected in pollen and nectar from seed-treated crops are generally too low to cause immediate bee deaths from acute poisoning. Neonicotinoid residues in nectar and pollen of treated plants often lead to long-term exposure of pollinators. Referring to TENNEKES (2010b) there is no safe level of exposure, as even tiny amounts of systemic insecticides can have negative effects in the long term. This is attributed to the fact that the damage neonicotinoids cause to the central nervous system of insects is both irreversible and cumulative. And as both receptor binding and the effect are irreversible, exposure time reinforces the effect (TENNEKES 2010a).

So far, a considerable amount of research has been carried out to investigate the sub-lethal effects of neonicotinoids on bees. Various recently published scientific studies suggest that in many cases low doses of neonicotinoids indirectly harm bee populations. The reported sub-lethal effects include a wide range of behavioural disturbances in honeybees:

- disorientation and difficulties in returning back to the hive (homing ability)
- reduced foraging efficiency
- impaired memory and learning
- failure to communicate properly with other bees in the colony
- reduction of breeding success
- decrease of metabolic efficiency
- reduction in disease resistance

Various studies on the reactions of honeybees to sub-lethal doses of neonicotinoids have shown that the insecticides negatively impact the ability of worker bees to forage and to communicate (cf. SCHNEIDER et al. 2012; HENRY et. al 2012; DESNEUX et al. 2007). Other research has demonstrated changes in the learning and memory abilities of bees upon exposure to low doses of neonicotinoids (BLACQUIÈRE et al. 2012). Sub-lethal doses of neonicotinoids can also lead to a disorientation of honeybees, causing them to fail to return to their hives (cf. HENRY et al. 2012; PESTICIDE ACTION NETWORK UK 2012). As colony growth strongly depends on food stores, the ability of honeybees to navigate to food sources as well as their remarkable ability to communicate is important for colony survival (cf. HOPWOOD et al. 2012).

Besides these behavioural effects, chronic exposure to low doses of neonicotinoids can also reduce the breeding success of exposed pollinator populations (cf. LU et al. 2012; WHITEHORN et al. 2012) and may lead to a neonicotinoid-induced decrease of the metabolic efficiency (HAWTHORNE & DIVELY 2011). In addition to that, studies performed by PETTIS et al. (2012) found a reduction in the disease resistance of exposed bees. Specifically, it has been found that pesticide exposure in honeybees induces increased levels of the gut pathogen *Nosema* (PETTIS et al. 2012).

As part of this research study, honeybee colonies were exposed during three brood generations to imidacloprid below levels considered to be harmful to bees[5]. The interaction between sub-lethal exposure to imidacloprid at the colony level and the spore production in individual bees of the honeybee gut parasite *Nosema* was clearly demonstrated: Infections with *Nosema* increased significantly in bees from hives treated with pesticides compared to bees from the control hives. Similar synergistic interactions between *Nosema* and neonicotinoids were published by ALAUX et al. in 2010. Also, VIDAU et al. (2011) proved that the exposure of honeybees to sub-lethal doses of thiacloprid significantly increased the mortality of honeybees that had been infected with *Nosema* before. Even the manufacturer Bayer's own booklet says that imidacloprid makes pathogenic soil fungi 10,000 times more dangerous for termites[6] (cf. TENNEKES et al. 2012). TENNEKES et al. (2011) also demonstrated that chemicals that bind irreversibly to specific receptors (neonicotinoids, genotoxic carcinogens and some metals) will produce toxic effects in a time-dependent manner, no matter how low the level of exposure. This goes along with the recent evidence of immune suppression in bees caused by neonicotinoids. According to TENNEKES et. al (2012) the neonicotinoid-induced decline of invertebrates leads to losses of birds, amphibians and bats who are feeding on them.

A study carried out by GILL et al. (2012) shows that chronic exposure of bumblebees to two pesticides (neonicotinoid and pyrethroid) at field-level concentrations affects natural foraging behaviour and enhances worker mortality, leading to considerable reductions in brood development and colony success. The researchers found that worker foraging performance (e.g. pollen collecting efficiency) was noticeable decreased with observed knock-on effects for forager recruitment, worker losses and overall worker productivity. Another important finding of the study was that combinatorial exposure to pesticides increases the tendency of colonies to fail.

Recently also two research teams of France and the UK published behavioural studies in the Science magazine about the effects of sub-lethal doses of neonicotinoides on honeybees and bumble bees (http://scim.ag/MHenry, http://scim.ag/Whitehorn). The studies carried out by HENRY et al. (2012) and WHITEHORN et al. (2012) clearly indicate that even very small quantities of the neonicotinoids thiamethoxam and imidacloprid adversely affect pollinator species and lead to weakening and decline.

HENRY et al. (2012) investigated the effects of low-dose, non-lethal thiamethoxam intoxication on the homing behaviour of honeybees. In particular the hypothesis was tested that sub-lethal exposure to this neonicotinoid indirectly increases the death rate of hives due to homing failure of foraging honeybees. In order to assess the navigation success of foragers, radio frequency identification (RFID) was used. Bees of three different colonies were intoxicated each day with a sub-lethal dose of thiamethoxam (1.34 ng in a 20 µl sucrose solution) and released up to 1 km away from their hive. The study clearly indicates that the homing success of foragers treated with non-lethal doses of thiamethoxam was significantly reduced compared to untreated foragers. While only 16.9% of the untreated bees did not find their way back to the hive, the percentage of the treated bees that did not return was 43.2%. An important finding of the study was also that the extent to which sub-lethal intoxication with thiamethoxam affects forager survival depends on the landscape context and on the knowledge of the bees about the area. Hence, when the homing task was more challenging, the mortality risk of the bees was higher, which demonstrates the problematic situation of solitary bee species that are probably less resilient to forager disappearance than honeybee colonies (HENRY et al 2012).

[5] Dosages 5 and 20 ppb imidacloprid
[6] Bayer Premise® 200 SC leaflet for termite control: http://www.elitepest.com.sg/brochure/Premise_200SC.pdf

Radio frequency identification (RFID) was also used by SCHNEIDER et al. (2012) in another research study, where effects of sub-lethal doses of clothianidin and imidacloprid on the foraging behaviour in affected bee populations were observed. Both neonicotinoids resulted in a significant reduction in the foraging activity within only three hours after treatment with ≥ 0.5 ng/ bee (clothianidin) and ≥ 1.5 ng/ bee (imidacloprid).

In the study carried out by WHITEHORN et al. (2012), the effects of the most widely used neonicontinoid (imidacloprid) on weight gain and the production of queens in wild bumble bee colonies of *Bombus terrestris* was tested. Colonies were fed pollen and sugar solution spiked with the substance at field realistic doses under laboratory conditions. As typical imidacloprid doses in nectar and pollen range between 0.7 and 10 µg kg^{-1} and bee colonies in agricultural landscapes are exposed to 2-4 week pulses of exposure to neonicotinoids during the flowering period of crops (cf. WHITEHORN et al. 2012), the tested colonies were fed with similar toxin levels during two different treatments[7] over 14 days mimicking bee exposure to imidacloprid in canola. "Control" colonies were provided with untreated pollen and sugar water. After 2 weeks under laboratory conditions, all colonies were released in the field where their performance was observed for 6 weeks. An important outcome of the experiment was that colonies fed with toxins gained less weight during the investigation period than the controls: At the end of the project the intoxicated colonies were about 10% smaller than those not exposed to the insecticide, which means that they gathered less food and produced fewer workers. The most striking outcome of the experiment was that the exposed colonies suffered a 85% decline in the production of new queens compared with the control colonies. This poses a problem insofar as bumble bees have annual life cycles and just the queens survive the winter to establish new colonies in spring. The results of the study thus clearly indicate that even trace levels of neonicotinoid pesticides can have substantial negative impacts on wild bumble bee populations (WHITEHORN et al. 2012).

Despite the alarming findings of these recent studies the results are discussed controversially. Bayer CropScience, the main producer of synthetic pesticides, still holds parasites and pathogens (and not neonicotinoides) responsible for the observed declines in honeybees (STOKSTAD 2012). According to BayerCrop Science, the studies used doses of imidacloprid and thiamethoxam that were higher than the concentrations present in crops under field conditions. In line with this conclusion are also the statements of the European Food Safety Authority (EFSA 2012a) and of the UK Environment Ministry DEFRA (DEFRA 2012).

The responses of the French Ministry of Agriculture and the national French food safety evaluation agency ANSES to the findings of the study performed by HENRY et. al. (2012) were very different. They revised the current approval for thiamethoxam products (the neonicotinoid used in that study). The latest news is that France is planning to suspend thiamethoxam insecticides for oilseed rape seed treatments (PESTICIDE ACTION NETWORK UK 2012).

[7] "Low" treatment: colonies were fed pollen and sugar water contaminated with 6 µg and 0.7 µg imidacloprid "high" treatment: colonies were exposed to twice the above ("low" treatment) levels

EFSA concluded that before drawing definite conclusions on the behavioural effects of sub-lethal exposure of foragers exposed to actual doses of the tested neonicotinoids (thiamethoxam, clothianidin and imidacloprid) - and the consequences for the bee colonies -, it would be necessary to repeat the tests with different exposure levels and/or in different situations (EFSA 2012a). According to TENNEKES (2010a) "time-to-effect approaches" (which provide information on the doses and exposure times) would be needed to have a better idea about toxic effects of neonicotinoids on beneficial organisms. They would be necessary because the consideration of toxic effects at fixed exposure times does not allow extrapolation from measured endpoints to effects that may occur at other times of exposure.

7. OUTLOOK

Although the effects of neonicotinoids on pollinator species are discussed controversially, recent scientific findings urge for an update of the current risk assessment scheme which should take new research results into consideration (particularly the sub-lethal effects on/ risks for wild pollinator species).

EFSA's intention to continue exploring the subject is thus a necessary next step, especially since EFSA has been requested by the European Commission to provide a concluding statement, with an updated risk assessment, about the neonicotinoids thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid and their effects on bees. In its latest scientific opinion on the adequacy of current risk assessment tests and decision-making for risks to bees and other pollinators, EFSA acknowledged that the existing risk assessment for these pesticides is inadequate and has to be revised (EFSA 2012b). EFSA specifically mentioned non-consideration of disorientation, larvae toxicity and the long-term effects of pesticides as shortcomings of the current risk assessment scheme. On the basis of the latest findings, EFSA is currently developing a new risk assessment scheme for plant protection products which takes into account their effects on bees (comprising *Apis mellifera*, *Bombus spp*. and solitary bees) and which will serve as guidance for applicants and authorities in the context of the evaluation of Plant Protection Products (PPPs) and their active substances under Regulation (EC) 1107/2009. In September 2012 a public consultation process (http://www.efsa.europa.eu) started on the draft guidance document, during which all interested parties are invited to submit their written comments.

At the same time, EFSA is currently elaborating an in-depth review of the acute and chronic effects of the above mentioned neonicotinoids on bee colony survival and development. In this context, effects on the behaviour of bees and on bee larvae are also taken into consideration. On the basis of this review, a re-evaluation of the authorisation of neonicotinoid pesticides will take place which is scheduled to be finalised by the end of 2012. In the re-evaluation process EFSA is supported by various Rapporteur member states: Spain for thiametoxam, Belgium for clothianidin, Germany for imidacloprid, Greece for acetamiprid and the United Kingdom for thiacloprid.

8. RISK MITIGATION MEASURES AND SUSPENSIONS

In Annex I to the Commission Implementing Regulation (EU) 540/2011 it is stated that risk mitigation measures for the substances acetamiprid (No 91), clothianidin (No 121), thiamethoxam (No 140), thiaclorid (No 92) and imidacloprid (No 216) should be applied where appropriate. In Directive 2010/21/EU, the following "specific provisions" are set out for clothianidin (No 121), thiamethoxam (No 140) and imidacloprid (No 216):

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

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

Member States shall ensure that:

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

Member States have thus implemented measures in order to avoid possible negative effects on bees from neonicotinoid pesticide use. These efforts are focused on minimising the exposure of bees to neonicotinoids in the process of sowing coated seeds with neonicotinoids as active ingredients.

An example of the planned continuous use of seed treatments with certain restrictions is given for Austria. The measures focus on minimising dust created during the sowing process and were introduced in Austria as a mandatory measure in 2012 (see Table 3).

Table 3: Examples of risk mitigation measures for neonicotinoids listed by the Austrian Agency for Health and Food Safety

Measures for coated corn seeds. The use of neonicotinoids is focused on the control of Diabrotica (corn rootworm).	Good quality of treated seed
	Obligatory use of a licensed adhesive agent for the seed treatment of corn
	Use of pneumatic sowing machines reducing drift
	Avoidance of dust drift into adjacent flowering vegetation during sowing
	No sowing of treated seed when windspeed > 5 m/s (18 km/h)
	Proper seed coating (limit set to 0.75 g dust/100.000 kernels)
	General restrictions of authorisations for maize seed treatment with neonicotinoids to control Diabrotica and wireworm only
	Sowing of treated seeds as treatment against Diabrotica during the first year of growing a maize crop (first-time maize or after crop rotation) is not allowed

Source: (AGES, 2012)

Furthermore, several European countries have (temporarily) suspended the use of certain pesticides in response to incidents involving acute poisoning of honeybees:

France: Sunflower and corn seed treatments of the active ingredient imidacloprid are suspended in France; other imidacloprid seed treatments, such as for sugar beets and cereals, are allowed, as are foliar uses.

Germany: The use of a number of seed treatment pesticides was temporarily suspended following an incident in May 2008 in which many bees were inadvertently poisoned. However, after investigating the factors contributing to the situation, Germany lifted the suspensions with the exception of the neonicotinoid clothianidin, whose use as seed treatment for corn remains suspended.

Italy: Certain imidacloprid and other neonicotinoid seed treatment uses have been suspended temporarily, but foliar uses are allowed. This action was taken based on preliminary monitoring studies in northern and southern regions of Italy showing that bee losses were correlated with the application of seeds treated with these compounds; Italy also based its decision on the known acute toxicity of these compounds to pollinators.

Slovenia: Neonicotinoid seed treatments for maize and oil seed rape (canola) were temporarily suspended. The suspension was based on poor seed treatment methods resulting in the release of dust during the seed sowing process. In August 2008, the suspension of oil seed rape seed treatments was lifted due to improved seed treatment methods and seed sowing equipment (EPA 2012).

Effectiveness of Measures

In all countries which have imposed restrictions on neonicotinoid seed treatments, widespread bee exposure to neonicotinoids continues via other approved uses. After imidacloprid seed treatment for sunflowers was suspended in France in 1999, the signs of poisoning continued also during 2000-2002. Bees in this region were still exposed to imidacloprid in maize pollen and to other systemic pesticides until they were suspended in 2004. Imidacloprid is also known to persist in the soil from seed treatment of other crops and could have been taken up by the following crop that was grown and that the foraging bees were feeding on (PAN UK 2012 a). These unintended continued exposures to neonicotinoids most probably diluted the intended harm reduction effect to be gained from the sunflower imidacloprid suspension imposed in 1999.

Italy suspended imidacloprid, thiamethoxam and clothianidin for maize seed treatment in autumn 2008, following the hypothesis that contaminated dust released from drilling machines during sowing played a large role in the observed hive losses. In the four years since Italy stopped maize seed treatment with neonicotinoids, evidence has shown that their bee populations are recovering. The results of the Italian monitoring network APENET show that bee deaths in maize growing areas were reduced to zero during the sowing period of 2009 and the following years. The losses during the winter also declined from 37.5% in 2007-2008 to around 15% in 2010 – 2011 (APENET 2011a). Stopping neonicotinoid seed treatments in maize certainly seems to have reduced damage from this exposure route, particularly acute toxicity linked with seed sowing (PAN UK 2012 a).

By monitoring the presence and population levels of the maize soil pests targeted by seed treatments APENET has shown that Italian Farmers using seeds not treated with systemic insecticides have not suffered negative effects on the yield and productivity of their maize crops (APENET 2011 b). APENET researchers conclude that banning maize treated seeds has seriously reduced bee mortality and that by rotating crops it has been possible to keep pests under control and to maintain yields.

Conclusion on the measures already taken and on available scientific results

The risk mitigation measures which are in place at EU and national level are limited. They are focused on reducing bee risks from acute poisoning but do not guarantee that harmful side effects will be prevented. According to recent findings, any measures that only reduce the application of neonicotinoids need to be treated with highest care. Several recent publications document the higher susceptibility of bees to diseases when exposed to different classes of neonicotinoids even at very low doses. This group of systemic pesticides has a long half-life in soils and active ingredients can be found in subsequent crops. Additionally, the degradation products can also show a pronounced toxic effect on bees. These facts clearly show that there is a risk associated with the application.

A communication from the European Commission (6th Dec 2010), also dealing with bee mortality, sees the need for more research projects to investigate honeybee health. In this communication it is pointed out that pesticides need to be approved at EU level only if they are safe for honeybees (HOMEPAGE EC 2010, Beekeeping and honey production).

As long as there are still open questions, the precautionary principle should be applied, as laid down in Regulation (EC) No 1107/2009 of 21st October 2009/(8) of the European Parliament and of the Council: *"The purpose of this Regulation is to ensure a high level of protection of both human and animal health and the environment and at the same time to safeguard the competitiveness of Community agriculture….*

The precautionary principle should be applied and this Regulation should ensure that industry demonstrates that substances or products produced or placed on the market do not have any harmful effect on human or animal health or any unacceptable effects on the environment."

According to chapter 2 (section 1, Article 4) which deals with the approval criteria for active substances, the following criteria need to be met:

"The residues of the plant protection products, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use, shall meet the following requirements:

(a) they shall not have any harmful effects on human health, including that of vulnerable groups, or animal health, taking into account known cumulative and synergistic effects where the scientific methods accepted by the Authority to assess such effects are available, or on groundwater;

(b) they shall not have any unacceptable effect on the environment".

Recent measures that still allow the use of neonicotinoids, like coated seeds, need to be scrutinised in detail as, according to recent scientific findings, these substances (especially imidacloprid, clothianidin and thiamethoxam) reduce the fitness of bees already at very low doses of exposure.

The active ingredients or their degradation products are persistent and accumulate in the environment (especially clothianidin and imidacloprid). Even a total ban on the most hazardous neonicotinoids cannot exclude future poisoning as a result of uses in the past and caused by the residues that have already accumulated in the environment.

Suggested future approaches

Honeybee losses and population declines are certainly multi-factored. The main factors are a reduction in adequate and good quality foraging sources, habitat degradation, reduced immune system defences to parasites and diseases as well as increased exposure to neonicotinoids and other pesticides and interactions between these stress factors (HOPWOOD et al. 2012; PETTIS et al. 2012; SPIVAK et al. 2010). Restricting or banning neonicotinoids will only address one of these factors, albeit a very important one which is increasingly linked to weakened colony vitality (PAN UK 2012 a).

Recommended measures for the better protection of bees are:

- Bans on certain neonicotinoids applications harmful to bees
- Multiple crop rotation, with maize only every second or (even better) every third year
- Searching for alternatives in pest control, e.g. pest control of the western corn rootworm (*Diabrotica virgifera)* with entomopathogenic nematodes (*Heterorhabditis bacteriophora*) instead of neonicotinoid seed coatings
- No spraying of neonicotinoids into flowering crops
- Preventive and non-chemical plant protection
- Cultivation of catch crops
- Improving the food supply for bees by multiple crop rotation, flowering strips and weeds
- Promotion of organic agriculture

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NOTES

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Existing Scientific Evidence of the Effects of Neonicotinoid Pesticides on Bees