EFSA Scientific Colloquium XVIII

On towards holistic approaches to the risk assessment of multiple stressors in bees

Parma, Italy, 15-16 May 2013

BRIEFING NOTES FOR DISCUSSION GROUPS

These briefing notes are prepared to provide participants with the relevant background information so as to be prepared for an interactive exchange of views and expertise during the Colloquium.

Background

Bees[1] play an important role in the ecosystem and the food chain through pollination, plant biodiversity maintenance and the provision of food and derived-hive products (for honeybees only) for human uses and therefore their protection is essential. The European Food Safety Authority (EFSA) which improves EU food safety and ensures a high level of consumer protection also need to protect bees and the ecosystem services they provide to humans. This task is currently undertaken by the Pesticides Risk Assessment (PRAS), Animal Health and Welfare (AHAW), Plant Health (PLH), Genetically and Modified Organisms (GMO), Scientific Assessment and Support (SAS) and the Emerging Risks (EMRISK) Units.

Given the consensus reached among scientists about the multifactorial origin of bee colony losses and the increasing body of scientific evidence showing the way stressors in bees may interact rather than acting solely, it is timely to assess the risks posed to bees and their services in a more integrated and multidisciplinary manner.

In line with the EFSA strategy which is to consider risk assessments (RA) in a wider integrated manner promoting in-house scientific expertise, tightening transversal collaborations across units and enhancing the inclusion of environmental aspects in the RA scheme, the EMRISK Unit of the Science Strategy and Coordination Directorate (SCISTRAT) whose task is to identify and coordinate horizontal scientific issues, established an internal task force to reinforce the protection of bees and their ecosystem services. In particular, the task force which includes representatives of the PRAS, AHAW, PLH, GMO, SAS and EMRISK Units and Communications Directorate has the objectives to identify cross-cutting issues, gaps of knowledge, research needs and recommendations based on the most recent developments made in the areas of bees and pollination, monitoring and risk assessment (EFSA, 2012). To perform this exercise, that is to review the state of the art of the work and research produced on bees in these areas, both inside and outside EFSA, the internal task force needs to liaise and exchange with stakeholders, from national, European and International Organisations.

This Colloquium titled "*Towards holistic approaches to the risk assessment of multiple stressors in bees*" will offer a unique opportunity to both international experts and EFSA for an open scientific debate on the most recent scientific progress made on pollination, monitoring and risk assessment of multiple stressors in bees along with current and futures challenges for food risk assessment in the European Union.

Objective

The objective of this Colloquium is to bring together international experts from different sectors for an open scientific debate on key issues related to the current state of the art on bees and pollination, monitoring, testing and risk assessment.

Organising Committee

Andrea Altieri, Jean-Lou Dorne, Tony Hardy (overall chair), Robert Luttik, Tobin Robinson (vice-overall chair), Agnès Rortais, Jane Stout (overall rapporteur).

EFSA (European Food Safety Authority), 2012. Inventory of EFSA's activities on bees. Supporting Publications 2012:EN-358.

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[1] All through the document, the term "bees" refers to honeybees, bumblebees and solitary bees.

DISCUSSION GROUP 1 - Protection of Bees and Pollination Services: Tools and Challenges

INTRODUCTION

Pollination by animals is an important ecosystem service, with 35% of global crop-based food production relying on animal-mediated pollination (Klein et al., 2007; Ollerton et al., 2011). Gallai et al. (2009) estimated the global economic value of crops pollinated by insects is € 153 billion/year, which is 9.5% of the total value of the production of human food produced for human consumption in 2005. In addition to pollination service, pollinators contribute to ecosystem function through benefitting the pollination of more than ¾ of all flowering plant species (Ashman et al., 2004; Aguilar et al., 2006; Ollerton et al., 2011). While pollinators are declining in various parts of the world (Biesmejer et al. 2006; Kluser and Peduzzi, 2007; Oldroyd, 2007; NRC, 2007), global agricultural systems are becoming more dependent on pollinators over time (Aizen et al., 2009) which may compromise agricultural and food production. Among pollinators, bees (both domesticated and wild species) play an important role in the ecosystem and the food chain through pollination, plant biodiversity maintenance and the provision of food and derived-hive products (for honeybees only) for human uses and therefore their protection is essential.

In order to design appropriate environmental risk assessment procedures, it is crucial to know what to protect, where to protect it and over what time period. In recent years, EFSA has broadened its scientific work towards the development of new risk assessment methods which take into account the environment (e.g. in the areas of plant protection products (EFSA, 2009), genetically modified organisms (EFSA, 2010a) and non-endemic plant pests (EFSA, 2010b, 2011)). In the area of pesticides risk assessment and bee health, EFSA has developed a methodology that allows deriving specific protection goals (SPGs)[2] for several organism groups applying the ecosystem service concept as a framework (EFSA, 2010c; Nienstedt et al., 2012). For bees, pollination, bee diversity and provisioning of food (honey and other bee-hive products for honeybees only) were identified as the ecosystem services to be protected (EFSA, 2012).

Pollination service and bee diversity are closely linked. Linear relationships were observed between crop yields and density of pollinators, e.g. in blueberries (Dedej and Delaplane, 2003), oilseed rape (Steffan-Dewenter, 2003), seed yields of flowering plants increased with abundance of flies (Clement et al., 2007). In support to these relationships, historical data showed parallel declines of pollinators and insect pollinated plants in Europe (Biesmeijer et al., 2006). Further testing and analysis at the species level showed that pollination service and bee diversity are synergistically associated through species interactions (Brittain et al., 2013; Garibaldi et al., 2013).

There is always a trade-off between the protection of such ecosystem services and the protection of plants. For example, while the protection of plants may be more important for a farmer than hive products, the protection of hive products may be more important for a beekeeper than the protection of plants and the society may give a high value to the protection of biodiversity. In order to take account these trade-offs, different protection goals for *in-field*[3] and *off-field*[4] need to be determined. For example, could less conservative protection goals be set *in-field* than *off-field*?

For the survival and development of honeybee colonies and effects on larvae and adult behaviour as listed in regulation (EC) No 1107/2009 (EC, 2009), it was suggested that attributes to be protected were defined. It was also proposed to include abundance/biomass and reproduction because of their

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[2] SPGs are defined in 6 dimensions: biological entity, attribute, magnitude of effect, temporal and geographical scale of the effect, and the degree of certainty that the specified level of effect will not be exceeded.

[3] "In field": a piece of land for cultivation with crops, managed and owned or rented by typically one farmer. The "in-field" also comprises a buffer strip that is a cropped or non-cropped zone of a defined width at the edge of a field which is influenced by the farmers action (e.g. spray drift). The buffer strip normally is enforced by authorities and underlies prescribed actions in orde to meet the "off-field" SPG. In addition, buffer strips may provide a recovery potential for the cropped area (if suitable off-field habitat is lacking).

[4] "Off-field": area surrounding a field: either (semi-)natural habitats with high ecological value such as hedgerow, grass strop, or simple structure (fence or a bare strip of land); normally no short-term changes in cultivation, in most cases not owned.

importance for the development and long-term survival of colonies. For optimal crop pollination and yield, some estimates on the numbers of honeybee hives and nesting females of solitary bees per ha are given in the literature (EFSA, 2012; Appendix A). The specific protection goal for abundance of bees could theoretically be based on these estimates – e.g. the application of a pesticide should not decrease the number of nesting females of solitary bees below these thresholds. However in reality the number of nesting solitary bees and the number of bee hives will vary greatly and therefore it would be very difficult to use these numbers directly in the risk assessment. As a surrogate, effects defined as percentage of mortality of bees were suggested (EFSA, 2012). In the total absence of pollinators, Aizen et al. (2009), estimated a total production deficit ranging from 3-5 % in the developed world and 8% in the developing world. However, determining an effect threshold on pollination service which should not be exceeded remains a challenge.

DISCUSSION POINTS

1. What needs to be protected in an agricultural context, *in-* and *off-fields* ?
2. What are the tools and challenges to assess impacts on pollination service from effects on bees ?
3. What impacts could be tolerated (i.e. on bees, crops and wild plants) over which spatial and temporal scales and what are the available methods to determine such impacts?
4. What are the mitigation measures to protect bees, pollination service and plants ?

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DISCUSSION GROUP 2 - Monitoring of Bee Populations and Stressors: Harmonisation of Protocols and Data Collection

INTRODUCTION

In the natural environment, bees (both wild and managed, social and solitary) are exposed to multiple stressors of various origins (biological, chemical, environmental and technological including management practices), which might act individually or in combination and cause species and population declines as reported in Europe (Biesmeijer et al., 2006; EFSA, 2008; AFSSA, 2009) and in other parts of the world (NRC, 2007). For honeybees, such symptoms were termed colony collapse disorder (USDA, 2012).

EFSA (2009) made a series of recommendations to harmonise surveillance of bee health including to "undertake specific studies that build on the existing work in progress to improve the knowledge and understanding of factors that affect bee health (for example stress caused by pathogens, pesticides, environmental and technological factors and their interactions) using appropriate epidemiological studies (case control and longitudinal studies)". Since then, worldwide, several surveillance programmes are currently monitoring honeybee mortality, collapse and weakening (e.g. the international COST network COLOSS-Monitoring[5]; the Bee Informed Partnership[6] in the USA; the EU pilot project for the surveillance of honeybee colony mortality from the European Commission[7]; DeBiMo[8] in Germany; APENET[9] and more recently BEENET[10] in Italy; WIIS[11] in the UK; etc.). There are also a few finalised and on-going research programmes, conducted at both local and global scales, for the monitoring of bee populations in general, and the testing of the factors involved in bee declines (e.g. FP6 ALARM[12]; FP7 STEP[13]). Such initiatives should be further encouraged and supported. Importantly, it remains true that wild bee populations and their interactions with stressors remain largely understudied in comparison to managed honeybees.

The COLOSS project, which focused on honeybees, concluded the followings:

- The mite *Varroa destructor*, in combination with viruses, is the main threat to honeybee colony survival in Europe,
- Interactions among parasites, pathogens, and pesticides can negatively impact the health of individual honeybees and,
- Gut parasites *Nosema* spp. can also affect honeybee health, but are not major stressors.

The "Bee mortality and bee surveillance in Europe" study (EFSA, 2009) identified a lack of comparable figures on honeybee colony losses, mainly because surveillance systems implemented in European Member States did not follow the same methodologies and were rarely applied to representative samples of the populations. The EU surveillance project that is currently in progress is a cross-sectional study that seeks to obtain comparable figures on colony losses across Europe. It also aims at estimating the prevalence of the major honeybee pathogens and investigate the relationship between these pathogens and colony losses or weakening during over-wintering or the production season. A longitudinal study taking

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[5] COLOSS: Prevention of honey bee Colony LOSSes at http://www.coloss.org/

[6] Bee Informed Partnership at http://beeinformed.org/

[7] 2012/362/EU: Commission Implementing Decision of 4 July 2012 concerning a financial contribution by the Union to certain Member States to support voluntary surveillance studies on honeybee colony losses (notified under document C(2012) 4396)

[8] DeBiMo : Deutsches Bienen Monitoring at http://www.staff.uni-marburg.de/~ag-biene/en/debimo.html

[9] APENET: Network for monitoring Bee mortality and colony loss in Italy at http://www.reterurale.it/apenet

[10] BEENET : bee national monitoring network

[11] WIIS: Wildlife Incident Investigation Scheme at http://www.pesticides.gov.uk/guidance/industries/pesticides/topics/reducing-environmental-impact/wildlife

[12] ALARM: Assessing Large Scale Risks for Biodiversity with Tested Methods at http://www.alarmproject.net/alarm/

[13] STEP: Status and Trends of European Pollinators at http://www.step-project.net/

autumn, spring and summer observations in Germany for 6 years was also able to give some quantitative estimations related to interactions between pathogens found within honeybee colonies (Hedtke et al., 2011).

Future epidemiological studies need to account for multiple and newly identified stressors, bee diversity (e.g. managed *versus* wild bee species), bee organisation (i.e. solitary *versus* social bees) and bee ecology/biology (e.g. habitat, foraging and home ranges, population dynamics, diet: specialists *versus* generalists, etc.). Longitudinal studies to investigate specific hypotheses about the interaction of stressors and their effect on bees (at the individual, colony and population levels) would increase our understanding of the changes in bee populations.

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DISCUSSION POINTS

1. Which hypotheses could be investigated in longitudinal studies, considering the most important stressors for bees under field conditions and those stressors which may act in combination?
2. Longitudinal study design would require a comparison between different exposure groups, which factors should be considered when selecting study sites?
3. Which indicators would be suitable to investigate these hypotheses (measurable, reproducible, repeatable, cost effective)?
4. Which indicators should be integrated into the large scale surveillance systems in order to feed the generation of hypotheses and to estimate the distribution of the tested factors?

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DISCUSSION GROUP 3 – Testing stressors in Bees: from Laboratory to Field conditions

INTRODUCTION

In the field, bees are exposed to a multitude of factors (pathogens, parasites, pesticides and some genetically modified (GM) plants, habitat fragmentation and change of environmental conditions or stresses such as poor nutrition), which might all together contribute to the decline of pollinators and to Colony Collapse Disorder (CCD) in honeybees. Decline of other bee pollinator species (i.e. bumble bees, solitary bees) was also reported in several regions of the world (Biesmeijer et al., 2006; NRC, 2007).

In recent years, comprehensive research efforts have been put into understanding honeybee colony losses but no single driver has yet emerged as a definitive and unique cause of the phenomenon (Neumann and Carreck, 2010). Also, there is no clear picture on which factors should be considered in a regulatory perspective and how these factors or the interactivity of them should become part of a regulatory assessment. Moreover, there is no clear picture on the testing methodology of these factors.

Standardised test protocols such as OECD 213/214 (OECD, 1998a, b) or OECD 75 (OECD, 2007) exist and are used for the risk assessment of potential chemical stressors (i.e. pesticides). According to these protocols, the use of 'healthy young adult bees' or healthy bee colonies in 'good condition' is recommended. Moreover, the test species used is currently restricted to the domesticated honeybee, *Apis mellifera*. Data are usually collected for typical measurement endpoints such as survival, development duration, percentage of individuals that reach a certain life-stage, weight or reproduction.

If a wider range of testing methodologies was available (i.e. more bee species, more bee stressors, and other endpoints than mortality or reproduction), our understanding of the main drivers of bee declines would be improved as well as our ability to mitigate the impact from these deleterious effects.

Recently, several new proposals for test protocols were published in this field (Aupinel et al., 2007, 2009; van der Steen et al., 2001; Ladurner et al., 2003, 2008; Gradish et al., 2012; Decourtye et al., 2010, 2011; Aliouane 2009; Schneider et al., 2012), but few useful steps towards the development of a set of harmonised, standardised and relevant test methodologies for use in the environmental risk assessments were achieved.

In a recent EFSA opinion on the science behind the development of a risk assessment of plant protection products on bees (EFSA, 2012), the magnitude of effects was defined as negligible if the natural background mortality, compared to controls, is not exceeded. An effect is defined as small if the natural background mortality is increased for example by a factor of 2 for a maximum of 3 days. Further work is needed to give recommendations on the deviation from the controls up to which an effect is still considered negligible. The current methods of field testing would need major improvements in order to detect for example an increase in daily mortality of foragers by 10 % with high statistical power. Based on expert judgement, it was considered that a small effect could be tolerated for some days without putting the survival of a colony at risk. However, it is not clear to what extent the strength of the colony would be affected. Further research (modelling) is proposed to clarify this question and to revise the proposal for the magnitude and temporal scale of effects.

One of the aims of this discussion group is to compile the available test methodologies and to assess their usefulness. A further point will be to discuss whether, and if so to which extent, observed effects in the laboratory can reliably predict potential adverse effects in the field (e.g. in terms of colony survival and development).

When extrapolating from laboratory to field, challenges are particularly important in the assessment of sublethal effects. In the case of pesticides, a recurrent question is whether sub-lethal doses of pesticides can be responsible for Colony Collapse Disorder (CCD) in honeybees and declines in non-*Apis* species. To estimate the impact of pesticides on bees, several studies have focused on the assessment of sublethal effects, including methodologies with physiological and behavioural endpoints (e.g. Henry et al. 2012; Schneider et al., 2012; Whithorn et al., 2012; Gill et al., 2012; Alaux et al. 2010; Genersch et al. 2010; Vidau et al., 2011; Wu et al., 2011, 2012). At several occasions, the importance of including sublethal effects in bee risk assessment schemes was highlighted. However, it is unclear whether sublethal effects might cause or not a decrease in the colony and population size and if they do, to which extent. It is also unclear how to establish a link to the protection goals as now defined in the EFSA Guidance Document (EFSA, in preparation).

Field studies increase our understanding of the impacts of stressors on bees and for that reason, good experimental designs are required. However, the current usefulness of isolated and unreplicated field studies are questioned since the extrapolation from one field to another may not be possible due to the high environmental variability and the importance of the environmental context. Moreover, the large foraging range of social bees and the problem of finding uncontaminated areas for controls make it difficult to draw strong conclusions from field studies.

DISCUSSION POINTS

1. Which are the most important stressors for bees under field conditions and how should bee stressors under field conditions be assessed in a regulatory risk assessment (e.g. which ones, separately or in combination, are the most relevant and should be considered)?
2. What are the available tests (strengths and limitations) to assess lethal and sublethal effects in bees in the laboratory and in the field?
3. What are the available methods to measure accurately bee mortalities in the field and what is the acceptable threshold?
4. Is it possible to extrapolate sublethal effects observed on bees in the laboratory (which ones) to effects under field conditions. Is it possible to quantify these effects? How?

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- **DISCUSSION GROUP 4 –** Risk Assessment of Multiple Stressors in Bees: From Mechanistic to Holistic Approaches

INTRODUCTION

Risk assessment of multiple stressors in honeybees, solitary bees and bumble bees is a complex issue since chemical, biological, nutritional, and physical stressors are involved. In order to develop appropriate methodologies for the risk assessment of such multiple stressors, a number of critical issues need to be addressed including an understanding of both the exposure and hazard (toxicity/pathology) dimension at the molecular, individual and ecological level so that their relative contribution to adverse health effects in bees can be quantified (risk characterisation).

In terms of chemical stressors, pesticide toxicity is the most studied amongst chemicals, and there is growing evidence that bees are sensitive to single and multiple pesticide exposure as discussed in the recent scientific opinion of the PPR Panel on the "Science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)" and in the report of EFSA's Bee Task Force on an "Inventory of EFSA's activities on bees" (EFSA, 2012a, b). Recent studies show that combined pesticide exposure severely impact individual- and colony-level traits in bees (Gill et al., 2012). The rationale for the particular sensitivity of honeybees, and Hymenoptera in general, lies in their specific toxicokinetic/metabolic profile with the lowest known number of copies of detoxification enzymes within the Class Insecta i.e cytochrome P-450, glutathione-S-transferases, carboxyesterases (EFSA, 2012a, Johnson et al., 2009). In addition, there is some evidence from the literature of synergistic effects between multiple pesticides and active substances applied in hives as medicinal treatments against *Varroa* mites (e.g tau-fluvalinate) while toxicity data of contaminants (heavy metals, mycotoxins…) in bees are almost entirely absent (EFSA, 2012a). The mechanisms of such interactions have a toxicokinectic basis involving inhibition or induction of either detoxification enzymes or transporters enhancing the toxicity of the mixture (LD50 decreases) (Jonhnson et al., 2012; Mao et al., 2011; Hawthorne and Dively, 2011).

Currently, full dose-responses for synergistic effects between potential inhibitors and different classes of pesticides/contaminants are rarely available for either lethal effects or sub-lethal effects in bees (*Apis* and non-*Apis*) and EFSA has recommended such studies should be carried out in adult bees and larvae. These will provide a basis to generate mode of action information for the chemical classes involved, take into account the dose dependency of the synergy, the magnitude of the interaction at concentrations of environmental relevance as well as both the maximum potentiating factor of the synergist and the concentrations for which no potentiating factor occur in the dose response curve (EFSA, 2012a, b). Another important challenge when dealing with multiple chemicals is the extrapolation from laboratory to field to investigate the impact on colony size, populations and success especially regarding sub-lethal effects; the extent of their impact on colony success is currently unclear as well as their relevance to establish protection goals (defined in the EFSA Guidance Document, EFSA, in preparation).

With regards to biological stressors such as diseases, there is some limited evidence that interaction between honeybee diseases and pesticide toxicity may have synergistic effects on bee mortality as investigated in *Nosema*, for sub-lethal doses of imidacloprid, thiacloprid and fipronil (Alaux et al., 2010; Vidaux et al., 2011). Another recent study concluded that tau-fluvalinate treatment increased bees' susceptibility to infection from deformed wing virus (Lock et al., 2012). These laboratory studies also raise the challenge of extrapolation to field conditions since comparable field studies have never been published and comparable infections under field conditions are very difficult, if not impossible, to achieve (EFSA, 2012a).

Nutrition is also a key variable affecting bee health, their sensitivity to chemical toxicity, resistance to disease and overall survival of bee colonies. In honeybees (*Apis mellifera*), pollen is the main dietary source of proteins, amino acids and lipids and is essential to adult bee physiological development and to their resistance to parasites and pathogens. Recently, the influence of pollen nutrients on the transcriptome of worker bees parasitised by *Varroa destructor* has been investigated in bees fed with either pollen or sugar. The authors found that pollen activated nutrient-sensing and metabolic pathways and had a positive influence on genes affecting longevity and the production of antimicrobial peptides. In contrast, *Varroa* parasitism caused development of viral populations and a decrease in metabolism through inhibition of protein metabolism and the effect was not reversed by pollen intake (Alaux et al., 2010b, 2011). Another recent study demonstrated, through an analysis of gene expression in bee midguts using northern blots, that honey, pollen and propolis induces detoxification enzymes in bees (CYP6AS), through the natural flavonoid quercetin and that mortality in bees exposed to the mycotoxin aflatoxin either consuming sucrose or high-fructose corn was higher compared with bees exposed to aflatoxin fed honey (Johnson et al., 2012).

Finally, physico-chemical factors such as temperature, moisture and dissolved oxygen have also been shown to impact on toxicity of chemicals with some limited evidence correlating an increase in pesticide toxicity in bees with temperature (Holmstrup et al., 2010).

The aim of this discussion group is to critically evaluate the molecular, ecological and holistic approaches available to investigate the relative contribution of multiple stressors to bee health.

DISCUSSION POINTS

1. Which mechanistic studies and analytical techniques are available to investigate the relative contribution of multiple stressors on bee health? Strengths and limitations should be included.
2. Which semi-field and field methods/approaches are available to investigate the relative contribution of multiple stressors on bee health? Strengths and limitations should be included.
3. Which holistic approaches including modelling can be combining with results of mechanistic studies, semi-field and field studies to improve our understanding of the effects of multiple stressors on bees? Strengths and limitations should be included.
4. What are the current data gaps and future research needs to investigate the effects of multiple stressors in bees?

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