Risk assessment of pesticides and other stressors in bees: Principles, data gaps and perspectives from the European Food Safety Authority ☆

Agnès Rortais [a],*, Gérard Arnold [b], Jean-Lou Dorne [a], Simon J. More [c], Giorgio Sperandio [d], Franz Streissl [a], Csaba Szentes [a], Frank Verdonck [a]

[a] European Food Safety Authority (EFSA), via Carlo Magno 1A, Parma 43126, Italy
[b] Laboratoire Evolution, Génomes, Comportement, Ecologie, Centre National de la Recherche Scientifique (CNRS) - Université Paris-Sud (UMR 9191), avenue de la Terrasse, 91198 Gif sur Yvette, France
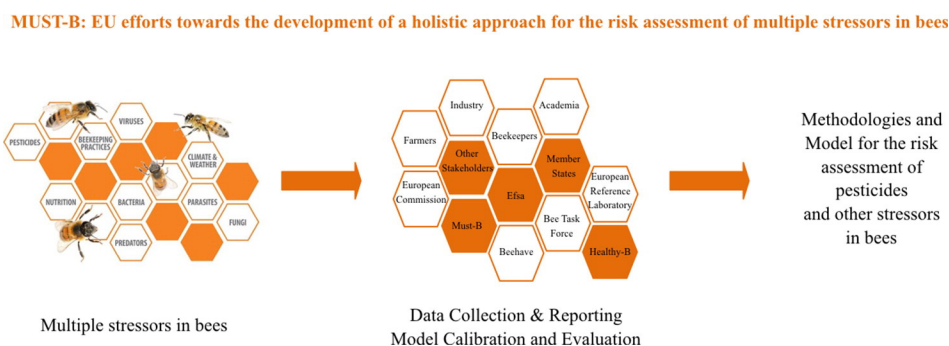[c] Centre for Veterinary Epidemiology and Risk Analysis, UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
[d] Department of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, 25123 Brescia, Italy

HIGHLIGHTS

- EFSA developed a risk assessment scheme for single/multiple chemicals in bees.
- Research is needed to fill gaps in risk assessments of multiple stressors in bees.
- A toolbox is available to assess honeybee health in field conditions.
- A model is designed to assess risk of pesticides and other stressors in honeybees.
- EFSA calls for an open-access database for risk assessments in bees.

GRAPHICAL ABSTRACT

MUST-B: EU efforts towards the development of a holistic approach for the risk assessment of multiple stressors in bees

Multiple stressors in bees

Data Collection & Reporting
Model Calibration and Evaluation

Methodologies and Model for the risk assessment of pesticides and other stressors in bees

ARTICLE INFO

ABSTRACT

Current approaches to risk assessment in bees do not take into account co-exposures from multiple stressors. The European Food Safety Authority (EFSA) is deploying resources and efforts to move towards a holistic risk assessment approach of multiple stressors in bees. This paper describes the general principles of pesticide risk assessment in bees, including recent developments at EFSA dealing with risk assessment of single and multiple pesticide residues and biological hazards. The EFSA Guidance Document on the risk assessment of plant protection products in bees highlights the need for the inclusion of an uncertainty analysis, other routes of exposures and multiple stressors such as chemical mixtures and biological agents. The EFSA risk assessment on the survival, spread and establishment of the small hive beetle, *Aethina tumida*, an invasive alien species, is provided with potential insights for other bee pests such as the Asian hornet, *Vespa velutina*. Furthermore, data gaps are identified at each step of the risk assessment, and recommendations are made for future research that could be supported under the framework of Horizon 2020. Finally, the recent work conducted at EFSA is presented, under the overarching MUST-B project ("EU efforts towards the development of a holistic approach for the risk assessment on MUltiple STressors in Bees") comprising a toolbox for harmonised data collection under field conditions and a

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* Corresponding author.

E-mail addresses: Agnes.rortais@efsa.europa.eu (A. Rortais), gerard.arnold@cnrs.fr (G. Arnold), Jean-Lou.dorne@efsa.europa.eu (J.-L. Dorne), simon.more@ucd.ie (S.J. More), Giorgio.sperandio@unibs.it (G. Sperandio), Franz.streissl@efsa.europa.eu (F. Streissl), Csaba.Szentes@efsa.europa.eu (C. Szentes), Frank.verdonck@efsa.europa.eu (F. Verdonck).

mechanistic model to assess effects from pesticides and other stressors such as biological agents and beekeeping management practices, at the colony level and in a spatially complex landscape. Future perspectives at EFSA include the development of a data model to collate high quality data to calibrate and validate the model to be used as a regulatory tool. Finally, the evidence collected within the framework of MUST-B will support EFSA's activities on the development of a holistic approach to the risk assessment of multiple stressors in bees. In conclusion, EFSA calls for collaborative action at the EU level to establish a common and open access database to serve multiple purposes and different stakeholders.

1. Introduction

The pollination of wildflowers and several key crops for food production rely on native and managed bees (Klein et al., 2007; NRC, 2007). In addition, managed colonies of honeybees (*Apis mellifera* spp.) represent an important source of goods and income with a yearly production of 1.6 million tonnes of honey and 65,000 t of beeswax (FAOSTAT, 2013). However, global declines in bee population pose threats to food security and the maintenance of biodiversity. For honeybees, large monitoring programmes indicate unprecedented rates of colony losses, in particular in Europe and North America (Laurent et al., 2015; Steinhauer et al., 2016), but similar observations, although less well documented, are being made in other parts of the world (IPBES, 2016).

Stressors affecting bees are multiple in nature and origin and these can be grouped into four broad classes: physical, chemical, biological and nutritional. Physical stressors are mostly governed by environmental changes (e.g. climate change, habitat fragmentation and destruction) while chemical stressors mostly include compounds of an anthropogenic nature (e.g. farming, urban/industrial/mining activities, beekeeping, gardening, etc.) as well as naturally occurring contaminants (e.g. mycotoxins, plant alkaloids, etc.). Biological stressors include bee pests and exotic diseases while nutritional stressors may be expressed as a change in the bee's nutritional status (e.g. proteins, lipids, sugars, vitamins and minerals). Both biological and nutritional stressors may be modulated by environmental changes and/or anthropogenic activities (e.g. an increase in bee pests and exotic diseases due to climate change and global trade; nutrition of bees related to resource availability in the landscape and beekeeping management practices). One of the challenges in environmental risk assessment is to include the combined effects of such stressors in risk assessment schemes (Holmstrup et al., 2010).

The European Food Safety Authority (EFSA) has the mandate to provide scientific advice related to food and feed safety issues in Europe. Bees represent a significant link in the food chain and ecosystem, and therefore it is critical that healthy stocks of bees are protected to enable the production of goods such as honey, pollen, propolis, royal jelly and wax (for honeybees), and the sustainable maintenance of the services that bees deliver (i.e. biodiversity and pollination). In this context, EFSA provides scientific advice and guidance on bee risk assessments related to a number of regulated stressors, that fall under its remit and are relevant to bees (i.e. plant protection products, animal diseases and pests and genetically modified organisms). In addition, EFSA provides scientific advice on peer-reviews of the risk assessment of all active substances used in plant protection products in Europe, which may include monitoring data. Furthermore, EFSA compiles and analyses in annual reports the information from the official controls of pesticide residues in food products (including honey) in European Member States (EFSA, 2015a). Further, it is under EFSA's strategic objectives 2016–2020 to prepare for future risk assessment challenges by generating, in cooperation with its partners, new scientific knowledge based on new developments and evidence (EFSA, 2015b). EFSA is proactively involved in the development of new risk assessment approaches with the integration of new scientific evidence regarding the impact of multiple stressors on bee health. However, developing new methods for the

risk assessment of those multiple stressors in wildlife populations is challenging given the high uncertainties with respect to how such assessments should be performed (Munns, 2006).

Within the past few years, EFSA has initiated a series of actions towards the development of a holistic approach for the risk assessment of multiple stressors in bees (EFSA, 2013a, 2014a). This work has been developed by an internal and multidisciplinary task force, called the EFSA Bee Task Force, gathering scientists from different units involved in bee assessments. The Bee Task Force started its work by making an inventory of all EFSA's activities and outputs dealing with bee risk assessment, risk mitigation and monitoring since the establishment of EFSA, in 2002 (EFSA, 2012). The Bee Task Force also organised a scientific workshop with all involved stakeholders to discuss the latest scientific developments on the risk assessment of multiple stressors in bees (EFSA, 2013a) and on approaches to broaden environmental risk assessments to account for multiple stressors (Devos et al., 2016). The conclusions of this event, together with a scientific report prepared by the Bee Task Force reviewing existing research projects in Europe and identifying knowledge gaps in this area, led to a series of recommendations (EFSA, 2014a) that were further reviewed and prioritised for future research under the framework of Horizon 2020 (EFSA and EC DG-AGRI, 2016).

The objectives of this paper are to review the principles of risk assessment of pesticides in bees, to discuss the data gaps and research needs for each step of the risk assessment process, and to provide an overview on EFSA's ongoing work to move towards risk assessment of multiple stressors in bees.

2. Principles of the risk assessment of pesticides in bees

Environmental risk assessment deals with the assessment of the risk(s) posed by a single or multiple stressors to which the environment or species under study may be exposed to. Frameworks for environmental risk assessment often use tiered approaches, which may use laboratory data at low tier and semi field to field data at high tier. In natural ecosystems or agro-ecosystems, bees may be exposed to a variety of stressors, whether of natural or anthropogenic origin including infectious agents, pests and predators (biological stressors), climate (physical stressors), habitat (nutritional stressors) and chemicals such as pesticides and environmental contaminants (EFSA, 2014a). Principles and methods for the risk assessment of pesticides in bees have been extensively reviewed both by EFSA and the US-EPA and the section below provides a brief overview of the key steps, namely problem formulation, exposure assessment and hazard and risk characterisation (EFSA, 2013b; EFSA PPR Panel, 2012; US-EPA, 2014).

2.1. Problem formulation and protection goals for bees and pollination

The basis of pesticide risk assessment schemes is to evaluate whether the use of a compound is acceptable in terms of its environmental impact and whether protection goals as outlined in the regulation are fulfilled.

In the pesticide regulation (EC, 2009), protection goals are broadly defined as the absence of unacceptable effects on the environment,

having particular regard to impacts on non-target species, including their behaviour, biodiversity and ecosystems. For honeybees, the regulation states that a substance should only be approved if it results in negligible exposure or has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.

For the development of a risk assessment scheme, it is necessary to define the protection goals more specifically in particular with regard to the magnitude of effects that are deemed acceptable. Therefore, EFSA has developed specific protection goal options for honeybee colonies (EFSA PPR Panel, 2012) based on an ecosystem service approach (EFSA PPR Panel, 2010; Nienstedt et al., 2011). The pollination services provided by a honeybee colony depend on the colony's strength which is defined as its size given by the number of individuals it contains (i.e. in-hive and forager bees). A review of existing methods to assess brood demography and colony size is provided by EFSA AHAW Panel (2016). Based on expert knowledge, EFSA attributed a large effect to a reduction in colony size of greater than one-third as this would most likely affect the viability, yield of hive products and pollinating capability of a honeybee colony. The definition of negligible effects was based on similar biological considerations and also by the possibility to distinguish it statistically from small effects. The effect classes between large and negligible effects were defined arbitrarily at even intervals between large and negligible effects (see Table 1).

Subsequently, the model of Khoury et al. (2011), which is a compartment model of honeybee colony population dynamics that explored the impact of different death rates of forager bees on colony growth and development, was used to link effects on colony size to forager mortality and viability of colonies (see Table 2).

Risk managers from Member States agreed in a dialogue organised by the European Commission on the protection goal of a negligible effect, which was defined as no reduction of colony size of >7% and forager mortality that should not be increased compared with controls by a factor of 1.5 for six days or by a factor of 2 for three days or by a factor of 3 for two days. The increase in forager mortality was used to derive trigger values which can be applied in the first tier risk assessment based on acute oral and contact toxicity tests as well as chronic oral exposure studies for adult honeybees (for details see EFSA, 2013b, appendices A and B).

When testing effects of pesticides on honeybee colonies, specific endpoints representing the main attributes of a healthy honeybee colony (i.e. queen presence and performance; behaviour and physiology; demography of the colony; in-hive products; disease, infection and infestation; see EFSA AHAW Panel, 2016) need to be selected and these effects need to be related to the protection goals. Indeed, given the wide range of possible endpoints to be covered, only a few, the most critical ones, need to be developed and feeding larvae is one of those. Nursing bees feed larvae with royal jelly produced by their hypopharyngeal glands (Crailsheim et al., 1992; Hrassnigg and Crailsheim, 1998; Maurizio, 1954). This organ is therefore essential for nurses to produce the food and perform their task and for larvae to feed and grow. For tests with effects of pesticides on hypopharyngeal gland development and consequently on larvae feeding (Hatjina et al., 2013; Heylen et al., 2010; Renzi et al., 2016; Smodiš Škerl and Gregorc, 2010), it was not possible to make such a quantitative link between the protection goal based on colony level effects and effect percentages observed in laboratory studies.

The level of protection is also determined by exposure considerations e.g. what percentage of exposure situations should be covered in the risk assessment. This includes for example the area that should be covered (e.g. the whole Europe, one of the EU regulatory zones (north–centre–south) or a Member State). The exposure assessment goal was proposed to cover a 90th percentile exposure situation for colonies at the edge of treated fields in the whole area where the compound is to be used. This ensures that the exposure in 90% of the colonies situated at the edge of a treated field will be below an exposure which could lead to a 7% effect on colony size. In the remaining 10% of the colonies, the magnitude of effects will depend on the margin of safety identified in the risk assessment (e.g. for compounds with a low toxicity to bees and a large margin of safety, it is unlikely that effects will be observed even if the 90th percentile exposure is exceeded; if the margin of safety is narrow, effects are likely when exposure exceeds the 90th percentile).

The honeybee example illustrates how general protection goals in the legislation can be transferred into specific protection goals in a transparent way and how these specific protection goals can be used to determine trigger values for a first tier risk assessment. The specific protection goal for other types of bees and their stressors need to be defined. The recent progress made on the development of specific protection goal options for environmental risk assessments of plant protection products, genetically modified organisms, feed additives and invasive alien species (EFSA SC Panel, 2016a) provides a framework to further harmonise protection goals for bees when considering additional stressors, other than pesticides, that are relevant for bees. In the case of invasive alien species, specific protection goals need to be interpreted as the entity that is impacted (by the invasive alien species), including its attributes and the spatial and temporal scales of effects which are defined case-by-case.

2.2. Exposure assessment

Exposure assessment has been defined in general terms by the World Health Organization as "the qualitative and/or quantitative evaluation of the likely intake of biological, chemical or physical agents via food as well as exposure from other sources if relevant" (FAO/WHO, 2008). In the case of environmental risk assessment for bees, exposure assessment deals with the evaluation of sources of exposure from the environment. For exposure assessment of pesticides in bees, occurrence data are combined with contact and dietary exposure and those are estimated separately using different approaches that are specific to different application methods (EFSA PPR Panel, 2012; US-EPA, 2014).

Individual bees or whole honeybee colonies may be exposed to pesticides via residues in different environmental matrices and bee products and through a number of means (e.g. for larvae via contact with contaminated food). Individuals leaving the beehive can be in direct contact with pesticides and residues can be carried back to the hive and contaminate the in-hive bees and the brood. As a result, foragers can be contaminated via consumption of guttation droplets from plants and/or by contact with dust drift from sowing treated seeds and/or via inhalation of high vapour pressure compounds during spray treatments. Finally, foragers and in-hive bees can be contaminated via consumption of nectar and/or pollen contaminated by spray treatments (see EFSA PPR Panel, 2012 for more details). Besides the application rate and application frequency, the means and magnitude of contamination are highly dependent on a number of elements such as the application methodology, the time of application and the treated crop. Landscape composition and other abiotic parameters like weather conditions can also have a significant effect on pesticide exposure in bees. In addition, in crop-dominated landscapes, non-cultivated plants represent an important source of pollen and nectar for bees (Long and Krupke, 2016; Requier et al., 2015). These matrices can be contaminated by a multitude of agrochemicals such as systemic chemicals (Botías et al., 2015) and pyrethroid insecticides targeting mosquitoes and other pests

Table 1
Definition of effect classes in terms of reduction of colony size.

Effect	Magnitude (reduction in colony size)
Large	>35%
Medium	>15% to 35%
Small	>7% to 15%
Negligible	3.5% to 7%

Table 2
Overview on magnitude of effects on forager mortality (increase in background mortality) and time to reach a certain colony level effect and whether the colony reaches the point where it may collapse after 50 days (i.e. when the total number of adult bees in the colony is below 5000 bees).

Multiple of background mortality (m)	Time to effects (in days)			Viable after 50 days?
	Negligible (reduction in colony size of ≤7%)	Small (reduction in colony size of ≤ 15%)	Medium (reduction in colony size of ≤ 35%)	
×1.5 ($m = 0.231$)	6	13	40	Yes
×2 ($m = 0.308$)	3	7	18	Yes
×3 ($m = 0.462$)	2	4	10	No

(Long and Krupke, 2016), and therefore represent a season-long route of pesticide exposure for honeybees. Finally, physical and chemical properties of the pesticide also have an influence on the magnitude and the length of the exposure and ultimately on the toxicity.

In its recent guidance document, EFSA (2013b) considered three types of application methods as the most important ones. These are spray application, seed treatment and granular application. In all cases, different compartments of the landscape are considered as potential sources of exposure; namely the treated crop, weeds in the treated field (except for seed treatment) and off-field (field margins and adjacent crops and/or plants). Moreover, the following crop (or in case of permanent crops, the same crop in the next year) can be a source of exposure to bees. This later scenario indeed becomes relevant when the pesticide is applied to an unattractive crop or to a crop in an unattractive stage (i.e. after the flowering), and when persistent residues can be taken up by the roots of an attractive crop in the following season. Off-field areas can be indirectly contaminated by a proportion of the spray liquid drifting away and depositing onto vegetated areas at the field margins or onto adjacent crops. Similarly, dust particles from solid formulations, including formulations used for seed treatment can be deposited to off-field areas (Long and Krupke, 2016).

With respect to how the bees take up the pesticides, EFSA PPR Panel (2012) distinguishes contact and oral exposures. The risk assessment for contact exposure focuses on forager bees visiting the treated field or the neighbouring off-field areas at the time of the pesticide application. The contact exposure is considered as a quick and acute event (especially for non-solid formulations) whereas the oral exposure can be continuous and long-lasting. Contaminated food (pollen and nectar) is stored and distributed in the hive. Therefore, the EFSA considers both acute and chronic oral exposures as relevant for foragers and in-hive bees. Additionally, a chronic oral exposure scenario is considered for the brood (larval stage).

Next to pollen and nectar, forager bees may collect water from the landscape. This water can also be contaminated by the pesticide. EFSA (2013b) distinguishes three different sources of water which can be potentially collected by bees; namely guttation fluid, puddle water and surface water. These water sources result in substantially different exposure scenarios. This is because the pesticide concentration in guttation fluid can be very high, but the collection and use of these droplets by bees is highly dependent on a number of biotic and abiotic factors (Joachimsmeier et al., 2011; Pistorius et al., 2011a, 2011b). Different crops shows variability in terms of frequency and intensity of guttation. Maize crop is considered the worst case scenario in terms of frequency, duration and intensity of guttation events and in terms of residue concentration (Pistorius et al., 2011a, 2011b). Broadly, younger crops show the highest residues in guttation fluid. Attractivity of guttation fluids for honeybees needs also to be considered when assessing exposure. Guttation fluids may be relevant for both acute (for foragers when they collect guttation water) and chronic effects (when guttation water is used to dilute honey, for example) (Reetz et al., 2011; Schenke et al., 2010, 2011). On the other hand, the collection of water from permanent water bodies is frequent but the concentration of pesticides in this water is usually rather low.

In addition to the above routes of exposure, the exposure of bees via honeydew, wax and inhalation may not be negligible and therefore may need to be considered. However, these routes of exposure are not entirely covered by the current risk assessment schemes.

2.3. Hazard and risk characterisation

2.3.1. Single compounds

First tier laboratory tests are the starting point for risk characterisation as they provide robust estimates of the intrinsic toxicity of a compound. In principle, hazard and risk characterisation of pesticides in bees aim to characterise the toxicity of the compound and the risks associated with its exposure in bees, respectively. Tiered approaches are applied. At low tier, laboratory-based data are collected on individual bees that are representative of different life stages (larval, pupal and adult) and castes (e.g. worker bees) whereas at higher tier, semi-field and field studies provide colony mortality data (US-EPA, 2014).

For hazard characterisation, standard test methods investigate acute effects on adult worker bees based on mortality after 48 h of a single exposure after oral or contact exposure (expressed as Lethal Dose for 50% of the individuals (LD_{50}) in μg/bee). During the 48 h test, observations may be prolonged up to 96 h if mortality continues to rise (e.g. OEPP/EPPO, 2010a; OECD, 1998a, 1998b).

In order to determine the toxicity for other life stages and other routes of exposure (i.e. residues in nectar, pollen and water), the recent EFSA Guidance Document (EFSA, 2013b) proposes to conduct chronic toxicity studies for exposure of adult bees over 10 days, and effects on larvae and brood care (e.g. via the assessment of the development of the hypopharyngeal glands). Furthermore, new OECD test guidelines have been elaborated for larvae development based on a single exposure to determine survival in larvae (OECD, 2013). A repeated exposure larvae test and guidelines for a 10-day chronic test for adult bees are also under development (OECD, 2016).

For risk characterisation, the quotient of toxicity and exposure estimates is then compared to trigger values. A trigger value can be defined as the ratio of the predicted exposure to toxicity (e.g. Exposure Toxicity Ratio or Hazard Quotients) whose exceedance indicates whether a specific protection goal is achieved or not. Trigger values are usually applied in tiered risk assessment schemes to decide in lower tiers whether further refinement of the risk assessment is required (EFSA, 2013b). Exceedance of such trigger values indicate that the protection goals are potentially not met and further refinement of the risk assessment is required.

The European pesticide regulation (EC, 2009) requires decision making on individual substances. Therefore, the current risk assessment aims to assess the effects of the use of one substance at a time. However, this does not fully characterise the real risk for honeybee colonies as they can be exposed to full treatment regimes in agricultural landscapes with different active substances which are applied during the growing season to a crop (Garthwaite et al., 2015). In addition, some compounds are applied in tank mixtures containing several active substances. Furthermore, honeybees are able to forage within several kilometres around the hive which increases the risk of experiencing multiple exposures and chemical mixtures in time.

2.3.2. Multiple compounds

Knowledge on the combined toxicity of multiple chemicals in wild bees is still limited. However, such scientific evidence in honeybees is

increasingly available, mostly from laboratory studies dealing with the acute toxic effects (LD_{50}) of compounds commonly found in bee matrices (i.e. pollen, honey and wax) namely insecticides (e.g. organosphosphates, neonicotinoids), fungicides, antibiotics and acaricides (e.g. varroacides) (ANSES, 2015; Johnson, 2015; Quignot et al., 2015; Spurgeon et al., 2016; Thompson, 2012). The mechanisms by which combined exposure to chemicals may exert their toxicity include dose addition, response addition and interactions. Interactions include synergy, for which an increase in toxicity occurs from the resulting interactions between the chemicals, and antagonism, for which a decrease in mixture toxicity is observed.

In this context, a number of studies have identified synergistic interactions, although in some cases, synergist concentrations are often orders of magnitude above concentrations of environmental relevance (EFSA PPR Panel, 2012; Johnson, 2015):

-Prochloraz, an imidazole fungicide and delthamethrin, a pyrethroid insecticide (Colin and Belzunces, 1992);

-Ergosterol-biosynthesis-inhibiting (EBI) fungicides, neonicotinoids, pyrethroids and organophosphates (Biddinger et al., 2013; Johnson, 2015; Johnson et al., 2013; Mao et al., 2011; Quignot et al., 2015; Thompson et al., 2014);

-Miticides: coumaphos and tau-fluvalinate (Johnson et al., 2009), coumaphos and tau-fluvalinate and in-hive antibiotics (oxytetracycline) (Hawthorne and Dively, 2011);

-In-hive antibiotic (oxytetracycline) and neonicotinoid insecticides such as imidacloprid, acetamiprid and thiacloprid (Hawthorne and Dively, 2011).

The scientific basis of such synergistic behaviour is often of a toxicokinetic nature. Since bees have the lowest number of a metabolising enzymes in the insect class (Claudianos et al., 2006), a compound in the mixture may inhibit or induce detoxification or induce the formation of a toxic metabolite resulting in an increase in toxicity. In particular, inhibition of cytochrome P450 enzymes or transporters such as P-glycoprotein involved in the detoxification of xenobiotics in the bee mid-gut are associated with such responses (Johnson, 2015; Hawthorne and Dively, 2011). For example, treatments applied by beekeepers to control a pathogen may interact with some agrochemicals applied by farmers and together act on the metabolism and immune system of bees (i.e. by decreasing the bee's ability to detoxification from xenobiotics and by increasing its susceptibility to infection by pathogens) (Boncristiani et al., 2012). However, it is important to note that studies showing these effects are mostly conducted under laboratory conditions and on individual bees, and need to be further explored in field conditions and at the colony level (Pettis et al., 2013).

In addition, other factors such as poor nutrition and absence of appropriate beekeeping practices may exacerbate such mixture effects (Johnson et al., 2012). As described above, for pesticide risk assessment, combined toxicity of multiple pesticides can be predicted and/or estimated through the concentration addition (CA) or independent action (response addition) (RA) approaches assuming similar or dissimilar mode of action respectively (Van Gestel et al., 2010).

In practice, for risk characterisation of combined exposure to multiple pesticides "mixtures" in bees, the Toxic Unit (TU) is considered as a conservative default approach. The TU is defined as "the ratio between the concentration of a mixture component and its toxicological acute (e.g. LC_{50}) or chronic (e.g. long-term NOEC) endpoint" The TU approach assumes dose (concentration) addition between the individual components of the mixture so that individual TUs can be added to calculate a TU of the mixture (EFSA PPR Panel, 2012; EFSA, 2013b; SCHER/SCCS/SCENHIR, 2012; Van Gestel et al., 2010). The TU approach is equivalent to the hazard index methodology used for risk characterisation of mixtures for human health (EFSA, 2013c).

In circumstances where evidence for synergistic interactions is demonstrated, the modified TU approach (or modified hazard index approach) can be applied using the magnitude of the interaction of the pesticide mixture under evaluation as a factor in the TU derivation. A methodology has been formulated by EFSA to deal with synergistic toxicity and include laboratory studies to characterise the full toxicity dose–response of the mixture in adult bees and larvae so that the magnitude of the interaction and synergy can be quantified in the risk characterisation (EFSA PPR Panel, 2012).

These principles may also apply to risk assessment approaches to deal with combined exposure to multiple stressors such as chemicals, contaminants, pathogens and pests but formalised approaches are currently lacking. Studies investigating the combined effects of pesticides and microorganisms are also increasingly available from the literature mostly for insecticides (neonicotinoids) and *Nosema* spp. (Alaux et al., 2010; Vidau et al., 2011; Gregorc et al., 2016). However, for other microorganisms, these studies are scarce (Quignot et al., 2015). Key data gaps and recommendations in this area are further discussed in Section 3.3.

3. Recent developments on the risk assessment of single and multiple stressors at EFSA

3.1. The EFSA guidance document on the risk assessment of pesticides in bees

Former pesticides risk assessment schemes for honeybees (SANCO/10329/, 2002; OEPP/EPPO, 2010a, 2010b) did not cover important aspects of the risk from pesticide applications (e.g. systemic compounds used in seed treatments). In particular, chronic exposure, sublethal and colony level effects are missing from the first tier assessments of these schemes (EFSA PPR Panel, 2012). Further, they did not include important routes of exposure such as contaminated off-field areas and water sources (EFSA PPR Panel, 2012).

The EFSA guidance (EFSA, 2013b) suggests a tiered risk assessment scheme which aims, at each tier, to evaluate whether specific protection goals, as agreed with risk managers, are met or not. The risk assessment covers exposure to residues in nectar, pollen and water and includes acute effects of oral and contact exposure and chronic (10-day) oral exposure of worker bees, effects on larvae and potential indirect effects on brood care. Specific trigger values have been developed for different life stages of bees and for different effect types in order to make the link to colony level effects. This has led to a complex first tier risk assessment. EFSA has created an Excel calculator to facilitate an easy and quick first tier assessment (EFSA, 2014b). One of the higher tier options is the refinement of exposure estimates based on measured residue levels in pollen and nectar and the sugar content in nectar. Such a refinement was missing from previous risk assessment, but now offers a cost and resource efficient intermediate tier before proceeding with semi-field or field effects studies.

The availability and quality of data posed some challenges for the development of the pesticide risk assessment scheme. In cases where insufficient data were available, a worst-case approach was followed. No risk assessment scheme could be developed to address the risk from exposure to residues in honeydew and only a tentative scheme could be developed for some sublethal effects such as homing behaviour.

The highest level of refinement of the risk assessment is the generation of field effect studies. However, the statistical power of the current test systems is usually low due to methodological shortcomings (e.g. unprecise tools to measure forager mortality and uncertainty around exposure related to how much nectar and pollen is actually collected and consumed from a treated field *versus* other sources) and variability between colonies. These shortcomings were described in detail by EFSA PPR Panel (2012) in Section 5.4.2 (e.g. colonies tested in plots size of 1 ha considering that bees on average forage over an area of 80 km^2 may lead to either an over or underestimate of the level of exposure of bees depending on the environment, i.e. whether it contains a high proportion of alternative treated or non-treated sources of nectar and pollen, respectively). Indeed, to ensure sufficient statistical power behind the detection of a 7% effect size change in honeybee colonies in field conditions, a high number of colonies would need to be tested

(Cresswell, 2011) and therefore new testing approaches such as simulation studies (Woodcock et al., 2016) and more robust tools to assess mortality are required.

3.2. The EFSA Scientific Opinion on the small hive beetle (Aethina tumida) and considerations on the Asian hornet (Vespa velutina)

According to a previous EFSA opinion (EFSA AHAW Panel, 2013), *Aethina tumida* (small hive beetle) is a bee-brood scavenger of *A. mellifera*, *Bombus* spp. (bumble bee) and Meliponini (stingless bees). Mature larvae leave the hive and burrow in soil to pupate. This coleopteran is a flying, predator that can survive and reproduce on a variety of ripe fruits, but not on vegetables, plants or flowers. Adults can detect airborne volatiles produced by *A. mellifera* and *Bombus* spp. and thereby can be attracted to the odour of bees and bee products. The pest is native to Africa but has spread to North America and Australia during the past 20 years. The larval stage of the pest is destructive to a bee population, whereas the adults have little impact. The larvae burrow through combs, eat honey and pollen, kill bee brood and defecate in honey, which subsequently ferments (see EFSA AHAW Panel (2013) and Cuthbertson et al. (2013) for further background information).

After the entry of *A. tumida* in Southern Italy, in early September 2014, in the Calabria region (Mutinelli et al., 2014; Palmeri et al., 2015), EFSA made practical recommendations to detect *A. tumida* and suggested risk management options to reduce its impact on honeybee colonies (EFSA, 2015c). Finally, it determined the pest survival, spread and establishment (EFSA, 2015d).

One of the main recommendations was to perform visual inspection of the colonies or commodities (i.e. queens and attendants, bee products to be used in apiculture, non-extracted comb honey and used beekeeping equipment) combined with the use of traps, baits and polymerase chain reaction analysis of hive debris to screen for the presence of small hive beetle adults, larvae, eggs and damage (see section 3.4.4 in EFSA (2015d) for a full review of available traps and baits). It was recommended that any observation or result of a screening test suggesting the presence of *A. tumida* should be confirmed by microscopy or polymerase chain reaction. Movement of an infested hive could promote the rapid spread of *A. tumida* over large distances. Modelling the natural spread of *A. tumida* ("distance-only" model) in the absence of any movement of the hives, suggested that the natural spread of the beetle alone would take more than a few hundred years to reach the Abruzzo region which is about 250 km further north from where the beetle has been originally detected. A model considering the ownership of multiple apiaries per beekeeper indicated that spread would be 10 times faster compared to natural spread of the pest. Opportunity maps showed that, once introduced, *A. tumida* could complete its life cycle in all European Member States between May and September. Detailed epidemiological studies would improve our knowledge of the survival, spread and establishment of the pest.

Treatments like heating, freezing and/or irradiation could be applied to eradicate *A. tumida* from non-living bee products and from used beekeeping equipment, but could not be applied to living material as these would kill bees and brood along with *A. tumida*. Prevention, control and/or reduction of *A. tumida* infestation in a honeybee hive while keeping the bees and/or brood alive, could be achieved using mechanical control and chemical treatments or applying good beekeeping practices.

It was recommended that restrictions on the movement of honeybees, bumble bees and commodities from infested to non-infested areas should be maintained until *A. tumida* is eradicated, to prevent spread of the pest. Increasing the frequency of visual inspections, preventing infestation using a fine mesh and issuing a health certificate for intra-Europe trade of queen bees, within 24 h before dispatch, could reduce the risk of *A. tumida* transmission via consignments. Maintaining good honey house hygiene and good beekeeping practices are the most important measures to control *A. tumida* where eradication is no longer the objective, given that no approved veterinary medicine is available in Europe.

The approach used by EFSA to assess the survival, risk of spread and mitigations measures for *A. tumida* could be modified and applied to other bee pests and predators such as the Asian hornet, *Vespa velutina*, which invaded France in 2005 and has subsequently expanded within Europe (Villemant et al., 2011a; Monceau et al., 2014) and is still expanding as shown with its recent detection in the Channel Islands-UK (PCN, 2016). Contrary to *A. tumida*, the population of *V. velutina* establishes outside the honeybee hive, in nests located in the surrounding of the hive, mostly in crown trees, but also in bushes, shrubs, natural or building cavities and ground holes (Villemant et al., 2011b). In Vespids, several control strategies have been tested such as the use of shelf-stable lures or baits combined with suitable toxins or pathogens, but with little success because of the structure of the colonies and the high Vespid population reproductive efficiency (Beggs et al., 2011). Indeed, as recently shown by an 8-year study conducted on nest distributions of *V. velutina* at a small scale in the southwest of France (Monceau and Thiéry, 2016), the species has not yet reached its carrying capacity. It is an opportunistic which establishes nests in areas where alternative food sources can be found. It may also be expected to adapt its behaviour and biology to the landscape and the environmental traits of its new habitat. Robinet et al. (2016) conclude that the most efficient control strategy remains the early identification of its presence in new areas by means of prediction and the identification of the precise location of the nests for their systematic destruction. Therefore, public awareness campaigns need to be raised and new detection techniques need to be developed. For example, the use of harmonic radars to detect nests in structurally complex landscapes (Milanesio et al., 2016) with greater detection of the hornet flying distances need to be further implemented. Another example is the potential development of sexual pheromone baits with a better understanding of the olfactory sensory system of the hornet (Couto et al., 2016). Finally, honeybees can develop efficient defensive behaviours against hornets, as shown in Asia between *A. cerana* and *V. velutina* (Ken et al., 2005; Tan et al., 2007, 2010, 2012a, 2012b, 2013) and in Europe between *A. mellifera cypria* and *V. orientalis* (Papachristoforou et al., 2007, 2011). It is assumed that these behavioural traits are a result of a co-evolution process between the prey and predator, which explains why *A. mellifera* has not developed an efficient defensive behaviour against its new predator *V. velutina* (Arca et al., 2014; Tan et al., 2007). However, recent findings on the development of cooperative behaviours between mixed species colonies to fight against *V. velutina* (Tan et al., 2012b) show that *A. mellifera* may eventually learn and develop a more efficient defensive behaviour against its new predator. With the recent addition of *V. velutina* on the EU list of invasive alien species (EC, 2016), funds and research efforts will be deployed to its study and control in the EU, but proper planning need to be coordinated at EU level (Monceau et al., 2014).

3.3. Data gaps and research needs at each step of the risk assessment process for the development of a more holistic risk assessment approach

The Bee Task Force together with stakeholders discussed and made recommendations on the steps forward for the development of a holistic approach for the risk assessment of multiple stressors in bees (EFSA, 2013a, 2014a). Knowledge gaps and recommendations were identified at each step of the risk assessment scheme, including problem formulation and protection goals for bees and pollination services, monitoring and exposure assessment, hazard assessment, risk characterisation and uncertainty analysis.

A summary of those recommendations is presented below and, whenever it applies, updates with new developments are provided.

• Problem formulation and protection goals for bees and pollination services

Current risk assessments are conducted on standard and surrogate test species. For bees, specific protection goals have been defined for

honeybees (*Apis mellifera*), bumble bees (*Bombus* spp.) and solitary bee (*Megachile rotundata* and *Osmia* spp.) for pesticides risk assessment (EFSA, 2012). Given the lack of data on mortality rates for bumble bees and solitary bees, specific protection goals for these species were extrapolated from those defined in honeybees by applying a safety factor in the risk assessment.

Considering the large diversity of bee species (about 20,000 known species and about 98% being solitary), the variability in traits between species, and the variability in species sensitivity to stressors as shown for pesticides (Arena and Sgolastra, 2014; Thompson, 2015), it is recognised that the specific protection goals for the test species may not be sufficiently protective for other bee species.

The compilation of extensive data on specific species traits varying from generic life-history traits to genetic traits, biomolecular traits and site-specific traits (e.g. actual population size and habitat preference) is required. These data would allow predicting responses of organisms to anthropogenic stressors in environmental risk assessments (EFSA SC Panel, 2016b; van den Brink et al., 2013).

• Monitoring and exposure assessment

In Europe and the US, there are several initiatives for the monitoring of honeybees and their exposure in their environment both at State (e.g. Genersch et al., 2010; Lodesani et al., 2012; Odoux et al., 2014; Porrini et al., 2013) and multi-States (e.g. Chauzat et al., 2014; Laurent et al., 2015; VanEngelsdorp et al., 2012) levels. These studies usually only select a subset of stressors that may affect bees and, they are usually conducted for a defined period, in line with resource availability. Importantly, it can be difficult to compare between studies, because of the use of different methods (e.g. beekeeper questionnaires and field observations *versus* laboratory detection analysis for specific chemicals and infectious agents). Therefore, in parallel to the development of monitoring plans for honeybees and other bee species conducted over the longer-term and with a wider scope, there is also the need for the development of guidelines to facilitate harmonised assessment of bee exposure to multiple stressors in the field, including standardised protocols, calibrated tools and validated detection methods. For honeybees data gaps were highlighted concerning exposure to pesticides by direct contact (via spray application and dust), by inhalation (for compounds with high vapour pressure) and by consumption (in weeds from infields, in field margins and off-fields; in pollen, wax, honeydew, guttation droplets). In addition, although new data were recently generated on residue levels in pollen and nectar over time (i.e. data from pesticide dossiers under data protection) or for dust drift deposition (SANCO/10553/2012, in preparation), residue data are still required on a wider range of chemicals (i.e. including veterinary products and contaminants and their metabolites) with single-residue analysis and low limits of detection and quantification for the most toxic compounds for bees. It was also noted that more information and data is required on bee biology (e.g. nutritive value of different types of pollen and beebread; sugar content in nectar carried by foragers; metabolism of xenobiotics in midgut; role of quercetin in detoxification processes; food intakes by other types of bees than honeybees, etc.) and behaviour (e.g. foraging preferences across different types of crops and landscapes).

Finally, given the breath of the data collection effort in terms of number of species, stressors, spatial and temporal scales and diversity of habitats to sample, there is a need to increase our monitoring capacity by promoting training, education and awareness on bees.

• Hazard assessment, risk characterisation and uncertainty analysis

EFSA's work has focused on chemicals that are regulated (i.e. plant protection products and some veterinary medicines such as acaricides used in agriculture). However, a large variety of pollutants are discharged into the environment from anthropogenic activities (e.g. agricultural operations, industrial activities, combustion of woods and fossil fuels, incineration of wastes, etc.), to which bees may be exposed. These pollutants possess different chemical and/or physical properties. For example, airborne particulate matter is a complex mixture of airborne chemical components, which may contain heavy metals (see Negri et al., 2015) - e.g. in form of mineralogical phases, linked to clay dust, etc. - or may carry pesticides (Tapparo et al., 2012), dioxin-like compounds and even biological agents harmful for the bee. Therefore, additional toxicity data are needed for different classes of chemicals, including contaminants (e.g. heavy metals and essential elements such as cadmium, lead, selenium and natural toxins such as mycotoxins and persistent organic pollutants), and their metabolites as well as for bees of different types (wild versus managed), sexes and castes (workers, larvae, queen and drones for social bees and adults and larvae of both sexes for solitary bees).

As suggested by the EFSA's Scientific Committee (EFSA SC Panel, 2009), full dose–response relationships to derive benchmark doses (and their limits) are required and could be linked to specific protection goals. For various types of bee species, those data would be used to generate species sensitivity distributions (Posthuma et al., 2002). Toxicokinetics and toxicodynamics data for the different chemicals and bee species should be determined under different temperature regimes and diets (e.g. sucrose *versus* pollen/nectar).

In order to collect such data, improvement of bee (laboratory and (semi)field) tests are required. For laboratory testing, lethal and sublethal endpoints, acute and chronic toxicity and toxicokinetics of single and multiple chemicals and contaminants are needed. Given the importance of queens and drones (for reproduction), the development of noninvasive tests is key. The inclusion of sublethal endpoints such as memory, orientation, communication, nursing, social immunity, food consumption, apoptosis, detoxification are documented in the scientific literature (see EFSA, 2013a, discussion group 3, for a review) but would need further validation before they can be routinely used. To this effect, some suggestions on how to include sublethal effects in a mechanistic population dynamic model were provided (EFSA, 2016a), but they still need to be further investigated. Those selected endpoints are on queen performance, brood rearing with the development of hypopharyngeal glands and homing ability of foragers. For (semi)field tests, although some recent and more accurate tools for field trails are being developed (Wang, 2015), improvements are still required on protocols to assess mortality rates and sublethal effects, to detect significant increases in daily foragers mortality and to include meaningful timescales (i.e. several brood cycles) and representative exposures (i.e. plot size).

Finally, new techniques such as omics and modelling (see Section 4.3 on the future development of a mechanistic model to assess risks to honeybee colonies from exposure to pesticides and other stressors) need to be further investigated to explore effects of co-exposures to multiple stressors in bees. Molecular markers can be used to better understand the underlying mechanisms under which bees are impacted by various stressors and show different sensitivities (Alaux et al., 2011). Nutrigenomics, transcriptomics and epigenomics may shed light on the role of the bee microbiome in bee nutrition, digestion and defense against pathogens (reviewed by Moran, 2015). Indeed, acute stress from the environment may affect the bee gut microbiome and its host which could be seen as a single biomolecular network, the "holobiont" with a "hologenome" (Negri and Jablonka, 2016). The importance of epigenetic processes on bee health warrant further investigation.

Modelling techniques can allow extrapolation from the individual to the population level by simulations of population dynamics processes and can increase realism with the inclusion of elements of the landscape by means of spatially explicit geographic information system mapping (Topping et al., 2016).

In addition, further development of methodologies to apply weight of evidence and uncertainty analysis to the risk assessment of multiple stressors in bees are needed. Tiered approaches may prove useful in

data rich and data poor situations under which deterministic (e.g. default values) and probabilistic methods could respectively be applied (EFSA, 2014a).

In conclusion, as described above, some of these recommendations are currently being addressed by EFSA (EFSA SC Panel, 2009; EFSA, 2016a) while others, following a process of prioritisation through expert elicitation knowledge (EFSA and EC DG-AGRI, 2016), will hopefully be addressed in the near future (SFS-16-2017, online).

4. Ongoing and future work on risk assessment of multiple stressors in bees: the MUST-B project

4.1. Overview

In 2015, EFSA initiated a large project called MUST-B "EU efforts towards the development of a holistic approach for the risk assessment on Multiple Stressors in Bees" to explore how multiple stressors and factors affect honeybee colonies in their natural environment. The project involves the multidisciplinary Bee Task Force and several groups of experts (i.e. BEEHAVE, HEALTHY-B and MUST-B).

The BEEHAVE model (Becher et al., 2014) was considered a very good starting point by EFSA for the design of a model to assess risks of pesticides exposure to honeybee colonies in the context of multiple stressors. BEEHAVE simulates the hive population dynamics by considering various factors that may impact colony activity and health (i.e. weather conditions, distance to patches, pollen and nectar availability influencing foraging ability, infectious agents such as the *Varroa* mite and some associated viruses, some beekeeping management practices, etc.). Therefore, the BEEHAVE group (EFSA PPR Panel, 2015) has evaluated the suitability of the BEEHAVE model for the risk assessment of pesticides in honeybee colonies.

The HEALTHY-B group created a toolbox to assess bee health in a holistic manner by listing and mapping indicators, factors and methods to determine the health status of honeybee colonies (see Section 4.2). The overarching MUST-B group includes the results and recommendations from the BEEHAVE and HEALTHY-B groups to further explore how multiple stressors and factors affect honeybee colonies in their natural environment. The initial MUST-B development includes the design of a predictive model to be used as a tool by risk assessors and managers to determine the risks posed by pesticides in honeybee colonies under different scenarios of exposure to multiple stressors (see Section 4.3). As a second step, an intensive focused field survey at a limited but representative number of sites in Europe (at least in the three EU regulatory zones) will be conducted to collect data for the calibration and evaluation of the model. At the interface between the model and the field survey, EFSA will use the list of indicators, factors and methods made by HEALTHY-B to generate a data model and a platform to store the data and feed the model.

MUST-B relies on strong networking activities with all involved partners (i.e. the European Commission, the European Reference Laboratory on honeybee health, the Member State authorities and a broad range of stakeholders involving beekeepers, scientists, industry and non-governmental organisations) (Fig. 1).

4.2. Toolbox with indicators, factors and methods to facilitate harmonised data collection in Europe

Weaknesses in the current honeybee surveillance systems in European Member States have been described (EFSA, 2008; Hendrikx et al., 2009). A European Reference Laboratory for honeybee health was subsequently established (EC, 2013a), and a pan-European epidemiological survey on honeybee colony mortality was conducted across 17 European Member States over a period of 2 years (Laurent et al., 2015). From this large dataset, EFSA contracted a statistical analysis where major lessons learnt were made in terms of training and data collection, in particular on the need to elaborate well-designed data models with controlled terminologies and strict business rules (Jacques et al., 2016). With its mandate to support European Member States in setting up an effective system of data collection and analysis at the Community level (EC, 2002), EFSA established an *ad hoc* working group of experts (i.e. HEALTHY-B, see previous section) to identify indicators, tools and methods to assess the health status of honeybee colonies in Europe in a harmonised and standardised way.

Based on a scoping of the scientific literature and subsequent discussion by experts representing different stakeholders, it was concluded that the characteristics of a healthy managed honeybee colony (in relation to the annual life cycle and geographic location) are that i) the colony has an adequate size and demographic structure; ii) the colony has an adequate production of bee products; and iii) the colony provides pollination services. These are characteristics of a healthy honeybee colony (but should not be seen as a definition) which lead to the identification of three overarching concepts: five colony attributes (referring to a managed honeybee colony: i.e. presence and performance of the queen; demography; in-hive products; behaviour and physiology; disease, infection and infestation; see blue boxes in Fig. 2), external drivers (referring to the colony habitat and management; i.e. resource providing unit, environmental drivers and beekeeping management practices; see green boxes in Fig. 2) and colony outputs (referring to the colony productivity in the perspective of human interest; see orange box in Fig. 2). Each attribute, external driver and colony output can be assessed via sets of indicators or factors (referring to abiotic or biotic components, respectively). The biological relevance of the listed indicators and factors was further assessed by the experts who ranked them according to their technical feasibility and priority for inclusion in field surveys implemented by beekeepers across Europe. For the latter, experts identified for each indicator and factor one or more "variables" and a specific "method". Finally, the working group provided guidance on the design for the analysis and field data collection with respect to assessing the health status of managed honeybee colonies (EFSA AHAW Panel, 2016).

The HEALTHY-B toolbox was shared with stakeholders for implementation (EFSA, 2016b). It is designed for all those involved in collecting, reporting and analysing data on bee health, to facilitate exchange of data for risk assessment and other purposes. It has the potential to evolve towards citizen (i.e. mainly beekeeper) involvement in the risk assessment process, which is part of EFSA's 2020 strategy. Efforts to improve data collection, reporting and analysis across Europe will facilitate risk assessment on bee health by national and European risk assessment bodies. The toolbox could be used to facilitate monitoring and comparison of bee health in time and space. The tools necessary to generate a "health status index" are described, to score the health status at colony or apiary level. Mapping the scores in both space (at regional or (inter)national level) and time would enable comparison and analysis of trends. Furthermore, the toolbox outlines methods on how indicators and/or factors could be identified that are key predictors of change in colony health status. Ideally, this would facilitate the detection of possible health problems at an early stage, so that a beekeeper could intervene before the colony dies. Furthermore, the toolbox is useful to develop a combination of methodologies required to take multiple stressors into account in future pesticide risk assessments. Besides the selection of indicators, factors and methods that could be inserted in a model and field data collections to inform and evaluate such a model (see Section 4.3), the toolbox could facilitate the harmonisation of bee health monitoring in relation to the pesticide post-marketing phase. A harmonised system regarding data generation and data collection, across Member States, would improve any analysis of the potential for impact on bee health at the European level. Based on the HEALTHY-B toolbox, EFSA could support to the Member States to harmonise this post-marketing activities and analysis of bee health.
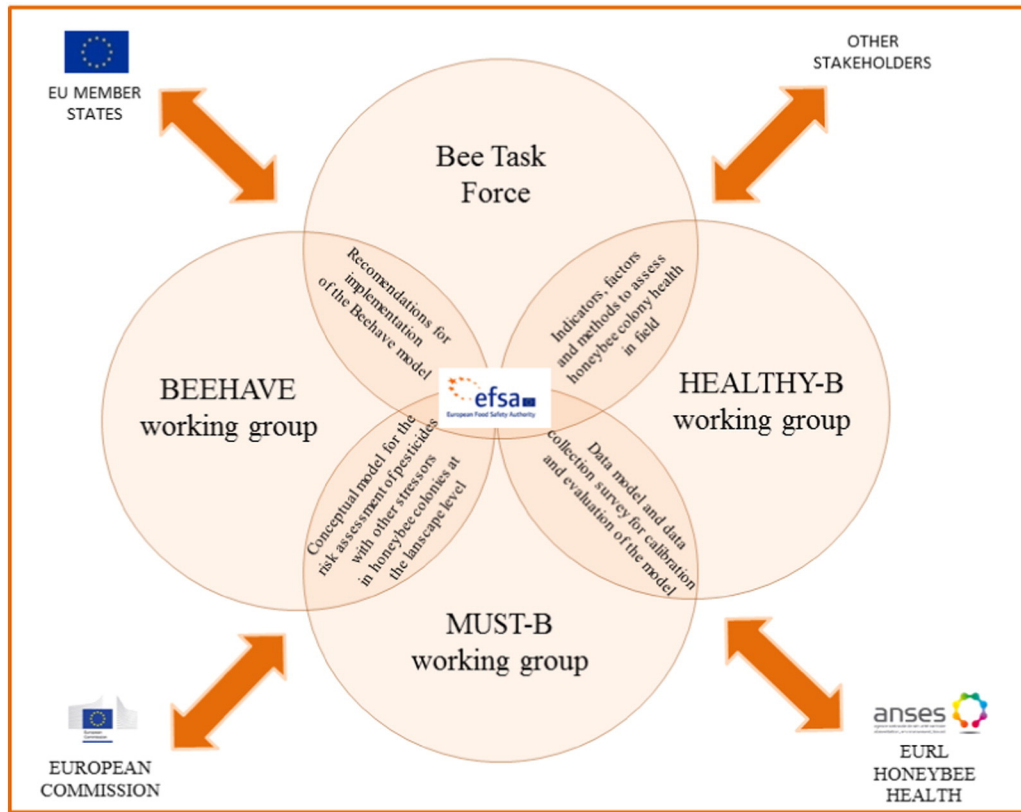
Fig. 1. MUST-B project: EFSA developments in interaction with external partners.

4.3. A mechanistic model to assess risks to honeybee colonies from exposure to pesticides and other stressors

Mathematical modelling seeks to simulate the behaviour of a system. They offer an opportunity to overcome many of the pitfalls posed by field experiments and measurements, in particular the difficulty faced when seeking to interpret the complexity of a honeybee colony and associated stressors. If realistic, in silico methods offer the opportunity to understand the impact of individual and multiple stressors on honeybee colony health (Becher et al., 2013; Devillers, 2014). Outputs
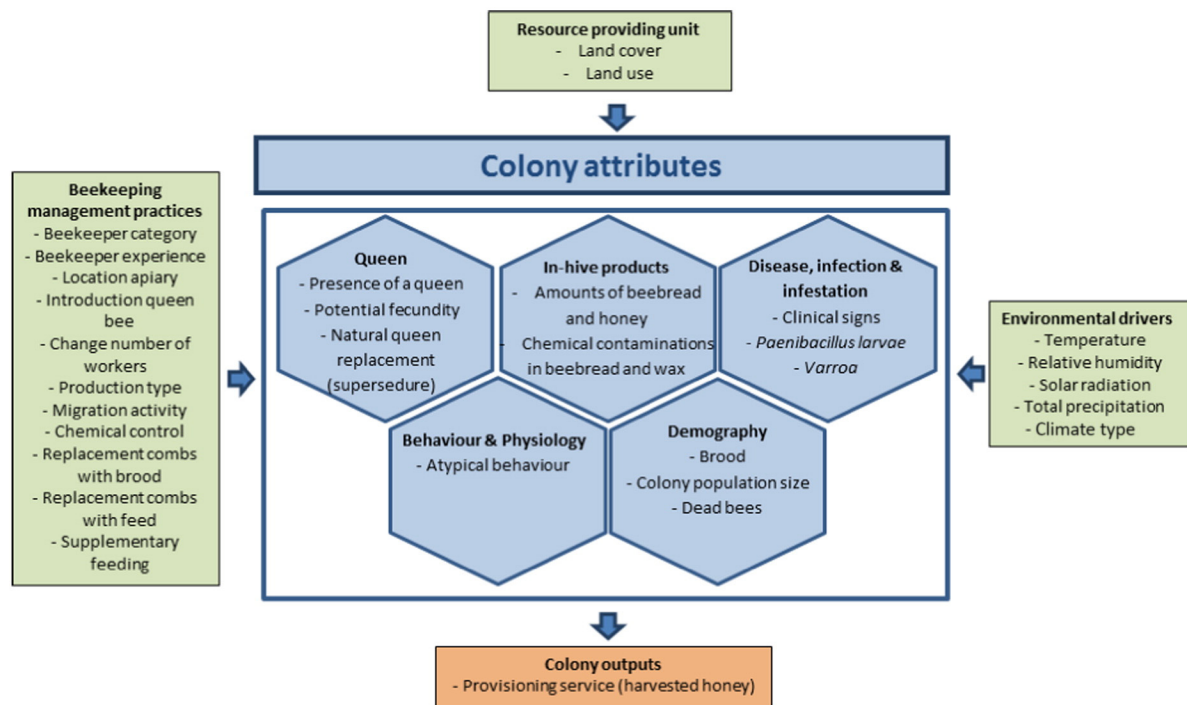

Fig. 2. Indicators and factors with high relevance, high technical feasibility and high priority as defined by HEATLHY-B to include in field surveys across Europe when assessing the health status of managed honeybee colonies in a holistic manner.

from such models provide insights into key colony attributes (e.g., colony size over time and forager mortality) relevant to defined protection goals.

In recent years, there has been substantial progress on mathematical modelling in this context, including the ongoing development (Becher et al., 2014) and application (Rumkee et al., 2015; Thorbek et al., 2016) of the BEEHAVE model, the first honeybee model to integrate processes both within the hive and in the landscape. The BEEHAVE model was recently reviewed by EFSA, specifically with respect to its suitability for use in a regulatory context (EFSA PPR Panel, 2015). BEEHAVE performs well in modelling honeybee colony dynamics, but is not yet usable in a regulatory context primarily because it lacks a pesticides exposure and effects module. A number of improvements were recommended, including additional modules to address chemical exposure and further important biological stressors such as *Nosema* spp., the incorporation of a realistic landscape for the assessment of multiple stressors and environmental factors, and opportunities for the model to be extended when new evidence is collected.

EFSA has recently completed a detailed technical report, outlining its vision for a mechanistic computer model for regulatory purposes to assist with risk assessment of pesticides in the context of multiple stressors and environmental factors on honeybee colony health (EFSA, 2016a). The report was developed by a multidisciplinary team, drawing on expert knowledge and a detailed understanding of current, rapidly expanding, scientific literature. Model development will be completed by a third party, under tender, in accordance with EFSA's opinion on good modelling practices (EFSA PPR Panel, 2014).

Conceptually, the proposed model can be considered a series of layers (Fig. 3). The first layer is represented by the base model. It is composed of three inter-linked modules: the foraging, the colony and the in-hive products modules which are connected to the landscape (second layer) comprising two other modules: the resource providing unit which is adapted from the concept of the service providing unit (Gilioli et al., 2014; Luck et al., 2003) and the environmental drivers modules. Service providing units highlight links between populations of species and ecosystem services and consequences of changes in population characteristics on service provisioning. The third layer comprises the beekeeping management practices, the biological agents and the pesticides modules.

- The *colony and in-hive products* modules. An individual-based modelling approach is proposed to simulate colony dynamics. The colony module relies on a physiological approach, and population dynamics are influenced by supply and demand for basic resources (related to individual bee energetics). Demographic, behavioural and physiological traits are defined by rate functions, and key state variables including colony size, demographic structure, quantity of food stores. Flows of nectar, pollen and water into the hive are either consumed or processed in the hive (nectar and water to honey; pollen to beebread). Besides food stores, in-hive products also include wax which is another important component of the colony considering the potential exposure of the brood during its development via contamination of the liquid surrounding the larvae (jelly) in brood cells through contaminated wax (see EFSA PPR Panel, 2012, table G1 for a review).
- The *foraging* module links the colony and in-hive products modules to the resource providing unit and environmental drivers modules. Foragers collect pollen, nectar and water (and other products such as propolis and honeydew which could not be included in the model because of lack of data), with foraging activity influenced by the colony needs, environmental drivers and characteristics of the resource providing unit.
- The *resource providing unit and environmental drivers* modules represents the structure and dynamics of the landscape, relevant to the availability of pollen, nectar and water, pesticide contamination and foraging behaviour.
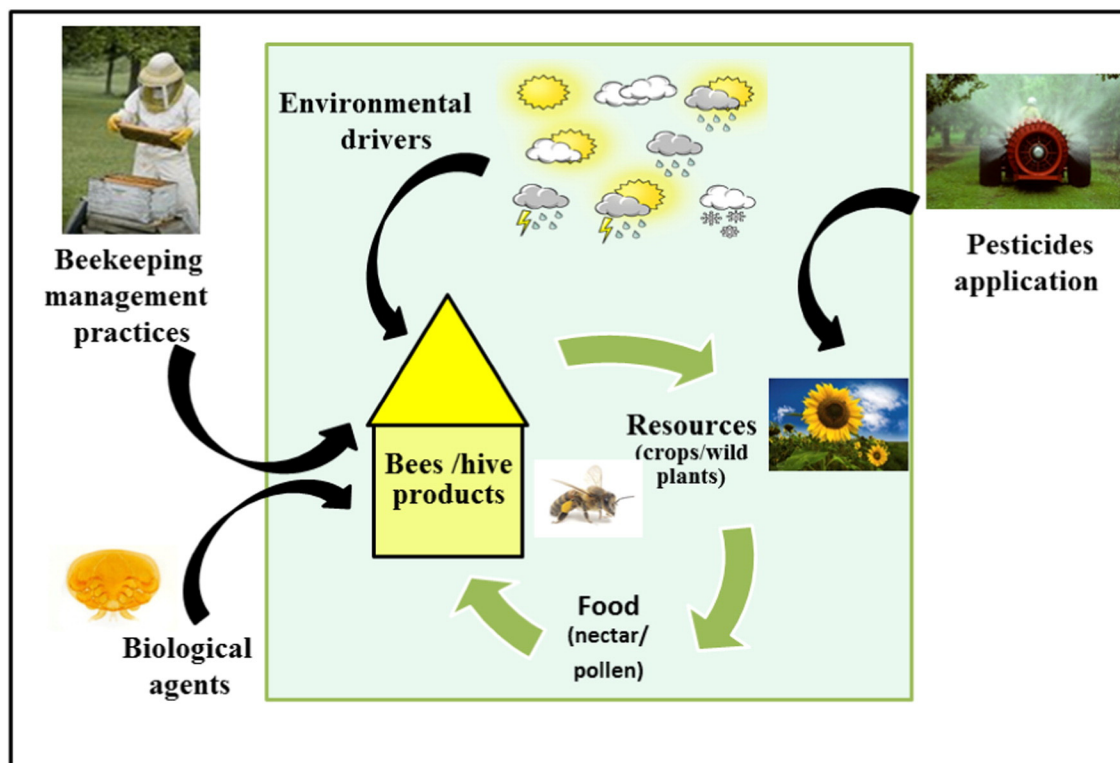

Fig. 3. Overall conceptual pesticides exposure/effect model showing the layered approach and linked modules.

Additional modules are then included to represent one or multiple stressors and factors on colony health:

- The *beekeeping management practices* module, representing a range of practices identified by HEALTHY-B (EFSA AHAW Panel, 2016 and Section 4.2). These factors will be modelled as scenarios using a quantitative approach (since there is very few data available) based on the "dose-effect" relationship to simulate impact on colony traits, including demography, physiology and behaviour.
- The *biological agents* module. This module represents a range of biological stressors, including microparasites and macroparasites. These stressors will also initially be modelled as scenarios, as above. In time, it is anticipated that some biological agents will be modelled as dynamic processes and that more agents are included.
- The *pesticides module* considers the flow of pesticides in the colony and in-hive products, the exposure by different bee categories (through contact and consumption for foragers, through consumption for all other categories), and of pesticide effects, including both lethal and sublethal for some specific endpoints (i.e. egg laying and homing behaviour).

4.4. The next steps: data collection for the calibration and evaluation of the model and perspectives

The model will primarily be used for pesticides risk assessment on honeybee colony health, but in the context of multiple stressors.

In time, model expansion is envisaged, including the potential for exposure to multiple chemicals, the addition of other biological agents such as invasive species (e.g. *V. velutina*) which may spread in Europe due to climate change and global trade (Barbet-Massin et al., 2013), the inclusion of multiple colonies in a complex landscape to simulate the effects of disease spread between colonies and among apiaries, shifting from scenarios to dynamic processes when considering multiple stressors, and, more broadly, incorporating new risk assessment methodologies, evidence and research data when they become available.

The model will need to be evaluated in at least three European regulatory zones (EC, 2009) comprising different environmental scenarios in terms of land cover, for the resource providing unit and environmental drivers. Consequently, post-model development evaluation will be important, based on field data collection. To aid this process, EFSA will provide the specifications for field data collection from defined sites throughout Europe. Close linkages are required between computer model development and field data collection, guided by the outputs of HEALTHY-B, which has identified robust indicators and variables for field data collection. Although the scope of this work is yet to be finalized, the model and these data may potentially be useful for other tasks, such as epidemiological studies to clarify the relative importance of different stressors.

Finally, with the above new collected evidence, under the MUST-B project, EFSA will prepare a scientific opinion for the holistic risk assessment of multiple stressors in bees. To further support this future task, ongoing work from EFSA regarding the development of new methodologies, in particular in the area of environmental risk assessment and evidence-based assessments (i.e. comprising an uncertainty analysis and a weight of evidence approach) will be considered. Weight of evidence has been defined by the World Health Organization as "a process in which all of the evidence considered relevant for a risk assessment is evaluated and weighted" (WHO, 2009). This approach provides a methodology to select, weigh and integrate the evidence in a systematic, consistent and transparent way to reach the final conclusions and to identify related uncertainties (EFSA SC Panel, 2015; SCENIHR, 2012). In this process, data from all sources and categories of literature should be considered for the risk assessment processes, as appropriate to determine their quality and relevance.

5. Conclusions

As recommended by EFSA (2014a) and recently recalled by the Members of the European Parliament at the occasion of 2016 European Bee Week (Penev, 2016), the multifactorial nature of the problem of bee losses requires several efforts and actions from all involved stakeholders. In addition, cooperation amongst European citizens, European and national institutions, beekeepers, farmers, researchers, animal health specialists, non-governmental organisations and industry experts need to be reinforced as well as public awareness on bees, beekeeping and pollination. EFSA's new efforts on the development of a tool for the risk assessment of pesticides in the context of multiple stressors will provide support to risk assessors and risk managers to make better informed decisions. In addition, the recent implementations of the Common Agriculture Policy, CAP 2014–2020 (EC, 2013b, 2013c, 2015) and the new Horizon 2020 (SFS-16-2017, online) call to invest research on data collection and methods development for the risk assessment of multiple stressors in different types of bees will provide solid support to decision-makers to reinforce legislation on bees taking into account the complexity of their environment (i.e. exposure from multiple stressors and effects at multiple temporal and spatial scales). Finally, to promote knowledge among all involved partners and to foster the exchange of research findings, expertise and best practices, EFSA recommends the development of an open-access and centralised database populated with data and methods to assess risks of single and multiple stressors on bees (EFSA, 2014a). Under the programme of MUST-B, EFSA is currently exploring ways to provide support for this development with the elaboration of a data model to be further populated and used by Member States. The coordination and management of such a database will need to be discussed with all involved stakeholders, at the European and national levels.

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