EVENT REPORT

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Workshop “The health status of a managed honeybee colony”

European Food Safety Authority

Abstract

The EFSA Panel on Animal Health and Welfare, together with the Panel on Plant Health, is working on the HEALTHY-B self-mandate that has the objective to map indicators and factors that could be included in large field studies to monitor the health status of a managed honeybee colony. The outcome of the work is a scientific opinion describing a framework that can be consulted by all stakeholders involved in measuring, analysing and reporting bee health in the EU. A workshop was organised with around 60 participants representing different stakeholder groups to identify relevant scientific evidence not yet included, to clarify the draft framework and to discuss potential harmonisation of measurements and reporting across the Member States. The outcome of the discussions and the collected information are very useful and will help the HEALTHY-B working group when finalising the draft scientific opinion. EFSA also explained how the HEALTHY-B framework will contribute to the progress of a wider EFSA mandate on multiple stressors in bees (MUST-B). Furthermore, the framework could be seen as a toolbox from which one could select what is needed and relevant according to the specific objectives of a given study assessing bee health. Further improvement of the ‘tools’ and gradual uptake by persons involved in bee health would provide opportunities to compare studies and would facilitate meta-analysis. Examples will be included in the HEALTHY-B scientific opinion to explain how the framework/toolbox could be used by different stakeholder groups.

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Key words: *Apis mellifera*, honeybee, health, attribute, driver, framework

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Table of contents

1. Background of the HEALTHY-B mandate

The way that stressors (mainly biological, chemical and environmental) affect honeybees (*Apis mellifera*) and contribute to poor health or losses in bee populations is poorly understood. The underlying mechanisms remain unclear due to the complex nature of the potential combinations and permutations of stressors acting independently or simultaneously and the effects of interactions between them. In this context, EFSA seeks to develop an integrated risk assessment approach for bees taking into account the multifactorial aspects of honeybee health status.

Information is already available in the published literature on attributes that influence and/or determine the health status of a honeybee colony, as well as approaches and methodologies that assess the health status of honeybees. However, there is a need for a harmonised framework defining the indicators that should be measured when assessing the health status of a managed honeybee colony in field surveys, that are agreed upon (and practical to implement) by beekeepers and other stakeholders, and feasible when applied at regional, national or European levels. The outcome of the work is the creation of a toolbox by EFSA that can be consulted by all the stakeholders concerned / involved in measuring, reporting and analysing bee health in the EU. It is certainly not a practical manual on how to keep a honeybee colony healthy.

The EFSA Panel on Animal Health and Welfare (AHAW), together with the Panel on Plant Health, is working on the HEALTHY-B self-mandate that contains four Terms of Reference[1] (TOR):

1. Identify and define the main colony attributes of a healthy honeybee colony.
2. Establish a framework that could be used to allow robust and harmonised measurement of the health status of a honeybee colony in field surveys.
3. Assess the availability of validated methods/tools for measuring indicators of honeybee colony health in field surveys.
4. Propose a methodological approach to allow robust and harmonised measurement and comparison of regional bee health status.

The HEALTHY-B mandate is one part of EFSA's multi-annual project on multiple stressors in bees (MUST-B) that has the objective to develop a holistic approach for the risk assessment of multiple stressors affecting the health of honeybee colonies. Only managed honeybees are considered since most knowledge and techniques are available for this bee species, *Apis mellifera*. However, a similar analysis for other bee species would be useful as bee diversity is very important, for instance for the delivery of pollination services.

For TORs 1-3, the organisation of a workshop on bee health was important to identify relevant scientific evidence that was not identified by the working group (WG), to identify unclear parts in the draft text and to discuss harmonisation of measurements and reporting. The workshop was announced on the EFSA website in December 2015 together with a call for interest for persons with relevant expertise who wanted to participate in the workshop. Around 55 experts submitted their interest to participate, from which around 30 were selected that fulfilled the eligibility criteria. Around 20 other experts were directly invited by EFSA, including the WG members. The workshop took place on 13-14 April 2016

2. Introduction and objectives of the workshop

The workshop started with a general session (i) to give an overview of the work done by the HEALTHY-B working group (WG) to reply to the TORs, (ii) to explain the objectives of the workshop and (iii) to present some lessons learnt and outcomes of other projects on bee health, to stimulate the discussions in the breakout sessions.

The work done so far was presented by the experts and EFSA staff members involved in the HEALTHY-B WG. A joint meeting with the MUST-B WG and interaction with hearing experts generated the view that a managed honeybee colony is considered healthy when, in relation to the annual life cycle of the colony and the region (i) it has an adequate size, structure and behaviour; (ii) it has an

[1] More information available at http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?2, last accessed on 25 April 2016.

adequate[2] production of bee products; (iii) it provides pollination services. This should not be seen as a definition of a healthy colony but as the basis of a hierarchical approach that has been developed to structure the scientific opinion. The highest hierarchical level consists of three overarching concepts which reflect the multidimensional characteristics of (i) a managed honeybee colony, (ii) its habitat and management and (iii) its productivity in the perspective of human interest; these three concepts are referred to as 'colony attributes', 'external drivers' and 'colony outputs', respectively. These concepts can be assessed via multiple sets of abiotic or biotic components, called 'indicators' (associated with colony attributes and colony outputs) or 'factors' (associated with external drivers). The identification of indicators and factors has been done by scoping of the scientific literature and input from experts of the WG and during the workshop.

Five colony attributes are identified as key components that should be analysed when assessing the health status of a honeybee colony:

- Queen presence and performance
- Demography of the colony
- In-hive products
- Behaviour and physiology
- Disease, infection and infestation

Three different external drivers are identified:

- Environmental drivers
- Resource providing unit
- Beekeeping management practices

Two colony outputs of the complex system described above are identified:

- The products harvested by the beekeeper from the honeybee colony
- The pollination services provided by the honeybee colony

For each of the colony attributes, external drivers and colony outputs, indicators or factors that could be monitored were identified and presented in mind maps. Then, the WG members scored the indicators and factors for their relevance to the health status of a managed honeybee colony, the technical feasibility in the context of a multifactorial field survey done by beekeepers/bee inspectors and their priority for inclusion in field studies across the EU. For the indicators and factors with a high score on all three criteria, analyses have been done on how they could be measured and reported in a harmonised manner in the EU.

The workshop participants received a draft text prepared by the WG before the workshop to consult all the mind maps and the corresponding description. A brief overview of the mind maps was presented at the beginning of the workshop.

The aim of the workshop was mainly to:

- Identify relevant scientific evidence that was not taken into account in the draft text
- Identify unclear parts of the draft text
- Discuss harmonisation of measurements and reporting

Breakout sessions were organised, according to expression of interest by the stakeholders, to discuss in detail the colony attributes, external drivers and colony outputs. These discussions were structured around the comments EFSA received from participants before the workshop, selecting those that would benefit from a discussion within a larger group of experts. EFSA also clarified that comments not requiring a group discussion were going to be examined by the WG; whilst editorial improvements would be done by professional proofreading before publication of the final document. Feedback from the breakout sessions was provided to all participants in a general session to give everybody the possibility to provide inputs on all topics covered during the workshop (see sections 3, 4 and 5).

A few presentations were given providing some lessons learnt and outcomes from other projects on bee health to stimulate the discussions. Tom Breeze (University of Reading, UK) highlighted the

[2] Sufficient to complete the annual life cycle at a given location.

importance of pollination in the production of crops and reported on studies indicating that pollination service supplies do not match the demand. He concluded that monitoring of pollinators and pollination would support decision making to maintain their ecosystem services.

Elke Genersch (Länderinstitut für Bienenkunde, DE) explained the assessment of bee pathogens in the German bee monitoring project, a study that started in 2004. The pathogens (e.g. viruses, bacteria) and pests (e.g. mites) included in the project were presented, as well as the methods used to measure them. One of the main challenges of the project was to find a balance between the costs (in terms of money, time and labour) versus keeping the beekeepers and scientists motivated. It was concluded that good data on bee health can only be obtained if there is an added value for beekeepers when they submit their data.

The COLOSS monitoring of honeybee winter losses was presented by Robert Brodschneider (University of Graz, AT). This project started in 2008 and is voluntary, anonymous and has a low threshold for beekeepers to submit data via questionnaires. A network has been created that is active in many countries in Europe as well as in the other continents. Although the sampling and accuracy of the collected data are limitations of the questionnaire-based approach, it brings value by generating hypotheses that could be further tested. COLOSS organises a meeting on 10-11 Sep 2016 in Cluj-Napoca (Romania) and invited all workshop attendants and EFSA to participate.

Mike Brown (National Bee Unit, UK) briefly summarised the work of the National Bee Unit (Animal and Plant Health Agency). Risk-based apiary inspections are performed on endemic diseases, exotics and contingencies. The programme is connected with research activities from other disciplines (e.g. veterinary medicine, ecotoxicology, environmental protection) and it aims to provide advice to policy. Detailed information is available on the web: www.nationalbeeunit.com. This programme is part of the UK's National Pollinator Strategy, which shapes the integration of the objectives with other bee initiatives and provides a basis for its continued implementation in the next years.

The assessment of demography indicators in field studies was presented by Sjef van der Steen (WageningenUR, NL) in reply to specific questions provided by EFSA before the meeting. Possible methods to measure the adult bee and brood population sizes were explained. Image analysis has a lot of potential to improve harmonisation across the EU. Smart phone applications could be developed to collect data directly from the field and, possibly, to send them to a database. However, implementation of such methods across the EU would require a standardised protocols and quality assurance tests.

3. Discussion of colony attributes

3.1. Queen Performance and Demography

During the first half of this session, the feasibility of assessing 'brood demography' and 'swarming rate' was discussed, as well as the relevance of variables such as 'brood pattern consistency' and 'presence of dead bees'.

The participants pointed out that including the brood pattern consistency as an additional variable would provide relevant information on the brood demography and on the queen fecundity. Assessing this variable would be feasible in field conditions because it would not require the use of specialized equipment and could be performed by a beekeeper during a routine hive inspection. However, it was stated that a clear definition of this indicator is needed, as well as a description of the method to assess it. It was suggested that the percentage of empty cells in the central area of both sides of a brood comb could be calculated using a rhombus (Collins, 2000), and a threshold of <10% of empty cells used as indicative of a good brood pattern consistency. The sensitivity of this method could be reduced in case the brood is infected with Varroa mites (by stimulating workers hygienic behaviour, leading to spotty brood pattern) and therefore the presence or absence of the mite should also be reported. The assessment of this indicator would require two people to increase accuracy of results and to reduce the duration brood is exposed to low temperatures. Regarding brood demography, it was agreed that assessing separately the amount of brood cells in relation to the development stage is only possible under research settings and therefore this practice should not be recommended.

The feasibility of assessing the colony swarming rate was also discussed. Although at this stage there is a lack of knowledge on the colony swarming dynamics and collecting data on this indicator would be useful, it was agreed that with currently available methods it is difficult to assess swarming rates in large field surveys. It would either require very frequent visits to the hive or use of automatic tools (such as sensors or scales), none of the options being feasible to implement in extensive surveys at this stage.

Regarding the relevance of the variable 'presence of dead bees', it was commented that it would be more useful to collect information on this variable outside the hive than inside the hive, because it is likely that the worker bees remove dead corpses from the bottom of the hive. It was pointed out that although the presence of dead bees could be very useful information in terms of pesticide exposure, for instance, collecting this information only three times per year may not be sufficient. Also the interpretation of the data could be difficult.

With regard to the use of sensor technologies to assess brood demography, it was concluded that while these tools could be very useful to monitor the colony dynamics on a long term basis, they first require further evaluation.

The second half of this session was dedicated to the colony attribute 'queen presence and performance'. The feasibility of assessing the indicators 'potential fecundity of the queen', 'queen longevity' and 'natural replacement of the queen' in field conditions were discussed. It was agreed that a qualitative assessment of the presence of 1 day old eggs to assess the potential fecundity of a queen is feasible in field conditions. On the contrary, it was considered that determining the number of patrilines to assess the same indicator is only possible under research settings (it would require microsatelite analysis). Therefore this variable could be included in the 'queen presence and performance' mindmap with a low score for priority to be included in field surveys across the EU. With regard to queen 'longevity' and 'natural replacement of the queen', it was stressed that it is only possible to assess these indicators by marking the queen. Therefore, marking queens should be strongly recommended in the scientific opinion.

A comment received before the workshop and not discussed in the workshop concerned the relation between pesticides and low sperm mobility/viability, because it was considered that this assessment would have a low technical feasibility in field surveys.

3.2. In hive products

The first part of the discussion mainly focused on suitable methods which may be used to monitor chemical residues. An expert claimed that residue analysis is not always the best method to assess health effects of chemical exposure, but no other validated approach seems to be available. It was mentioned that the objective of the HEALTHY-B toolbox is to measure multiple indicators and factors and to combine them in the health assessment of a colony. Analysis of the data could then test if there are associations or not (e.g. between pesticide exposure in bee matrices and abnormal behaviour). The probability to find residues in bee matrixes is highly dependent on time of sampling, weather and climatic conditions. Sometimes no chemical traces are found and the meaning of low residue levels is not clear. Therefore, assessing multiple indicators and factors at the same time is required. It was pointed out that an available source of data is the Residue Monitoring Plan that each MS has to provide to the EC. The major issue is that information is not always accessible. The importance to include in the assessment also non-authorised products was also raised as some of them are used.

Then the discussion turned on the relevance of chemicals to bee health. On this purpose, the following classes of chemical residues were discussed by the participants:

- Veterinary medical products: This class of chemicals is used as treatment against pests and diseases. However, scientific evidences showed that those substances have negative effects on bee health and thus need to be continuously monitored.
- Antibiotics: scientific research showed no direct negative effects on bee health so far. This class of chemicals is banned in Europe for use in bee colonies and its presence indicates deficient beekeeping management practices (BMP). The fact that its use in bee colonies is forbidden will prevent also beekeepers to report its use.

- Heavy metals: negative health effects of those compounds are mainly relevant to long- living animals. The participants, having considered honeybees' short lifespan, stated that heavy metals were not relevant for honeybee health. However, the importance to monitor heavy metals was highlighted as they may be found in bee products intended for human consumption and may cause concerns for human health especially if they accumulate to a toxic concentration level. The EU legislation on lead concentrations in honey was mentioned.
- Microencapsulated pesticides: the major issue pointed out by participants was that foraging bees may confuse pesticide capsules for pollen grains and store these in the hive. Concerns arose among experts on possible long-term effects at colony level when capsules break down. The lack of knowledge on this topic was identified as a research gap.

The second part of discussion focused on the importance to analyse bee matrices in order to assess exposure of bees to chemical residues. The importance of analysing beebread was highlighted as it is an important matrix to assess pesticide exposure for both lipophilic and hydrophilic compounds, and it represents an important source of exposure for honeybees. More research is required to better understand how pollen fermentation may affect pesticide residues in beebread. The participants considered measuring chemical residues in wax as highly relevant, because lipophilic compounds are highly persistent in this matrix and this feature may be used to assess in-hive chemical exposure history. However, due to its high composition in fatty acids it is mainly important for lipophilic compounds. With regard to honey, the participants pointed out the relevance of measuring chemical residues in this matrix (mainly hydrophilic compounds), as it is the major source of exposure for honeybees. In relation to other matrices, it was mentioned that the assessment of chemical residues could be done only in a research setting.

During the discussion, participants reached an agreement on proper timing of sampling which has to be similar across Europe and aligned to seasonal and climatic variations. It was proposed to proceed with sampling on 3 specific periods: after winter (1-2 weeks after flowering of *Salix* spp. and before first major nectar flow), during summer (active beekeeping season) and before winter (when colony is preparing for winter). After the workshop, it was found out that *Salix* spp. are present in large parts of the EU but not everywhere. Therefore, it might be better to define 'after winter' as 1-2 weeks after bees start foraging, but before the first big nectar flow. Considering the variability of the active season due to the different climatic zones in EU, the precise choice of the dates should be made at the national/local levels. It is clear that sampling and reporting more frequently will enrich the dataset.

The final part of the discussion focused on the technical feasibility of quantifying in-hive products. Participants clearly stated the difficulty of representative sampling and quantifying beebread. Therefore, it was proposed to use visual estimation and develop a proper category scale to quantify beebread. One comment that was not addressed during the discussion was about how to account for the variability in quantity and quality of beebread stored in each cell. It was also stated that approaches to measure quality and amount of beebread are feasible only under research settings and probably would not impact significantly on the final estimation of beebread amount and pesticide concentration. Regarding nectar and honey, participants clearly stated that quantifying them separately it is not feasible as the process that leads to the ripening of nectar to honey is a continuous process. Therefore, it is not possible to clearly define at which stage nectar becomes honey. Participants suggested quantifying both honey and nectar together by weighing supers or by visual estimation per frame. Furthermore, it was stated that when measuring in-hive products, it is important to consider BMPs as they play a major role on the amount of in-hive products.

All participants agreed that digital photography combined with image analysis or the use of other automated methods have a huge potential for harmonised collection of data in a continuous and accurate manner. However, there is a need for further development and validation before it can be used in a similar manner throughout the EU.

3.3. Behaviour and physiology

During this session the participants discussed the relevance and/or the technical feasibility of the indicators 'abnormal behaviour', 'colony foraging activity' and 'calmness', and the rationale for adding the variable 'presence of molecular markers' in the indicator 'immune response'.

With regard to 'abnormal behaviour', it was highlighted by the participants that assessing this indicator in quantitative terms (% of bees showing abnormal behaviour) may be very difficult, because it implies that the colony size is previously determined. Thus it was suggested to assess this indicator in a qualitative way (presence/absence of bees displaying abnormal behaviour). Moreover, due to the fact that characterizing abnormal behaviours in a narrative way can be difficult, it was suggested to create an online library of videos showing bees displaying abnormal behaviours to facilitate its recognition.

Regarding the 'colony foraging activity' indicator, the participants stressed that it is very difficult to get harmonised data on this indicator across Europe because the foraging activity can be affected by many factors, such as climate, season, weather, stores inside the hive and temperature, among others. As for the number of dead bees, this indicator could be useful to detect abnormalities at the moment of the field survey. Automatic tools under development to monitor the number of bees that leave and enter the hive are promising, however it was also acknowledged that the use of this type of technologies is currently not feasible to apply in extensive data collection exercises. It was suggested to use the honey yield and pollen composition as a proxy for the colony foraging activity. According to the participants, this would provide more accurate and comparable data. To have a complete picture of the colony activity dynamics it would however be necessary to collect data on this parameters (honey harvested by the beekeeper, honey in the nest and pollen composition) on a frequent basis.

The relevance of the indicator 'calmness' was also discussed. It was pointed out that this indicator is mainly influenced by the bee subspecies present in the colony, not being specific of healthy colonies. Hence it was suggested to decrease the score of this indicator (Low relevance to bee health).

One of the comments previously provided by some participants was the possibility of adding the variable 'presence of molecular markers' to assess the indicator 'immune response'. Nevertheless some participants stressed the fact that the bee immune response pathways are currently not well understood, and therefore more research would be needed to fully understand the relevance of this information. On top of this it was also considered that assessing this variable in extensive field surveys would not be feasible at this stage due to the laboratory tests it requires.

3.4. Disease, infection and infestation

This session started with a discussion on a flow chart that describes the steps of assessing the presence of disease, infection and infestation of a colony. It was concluded that the layout of the chart had to be improved. Then, the methods for testing for *Varroa*, *Paenibacillus larvae* (causative agent of American Foulbrood (AFB)) and *Melissococcus plutonius* (causative agent of European Foulbrood (EFB)) were discussed. The importance of Deformed Wing Virus (DWV) for honeybee health and the relevance to systematically assessing it in field conditions was debated, as well as the existence of data on a threshold that relates the number of *Nosema* spores in the hive and the presence of clinical signs in adult bees.

In relation to *Varroa* quantification, the participants were not aware of additional scientific evidence that was not yet identified by the WG. The alcohol method was agreed as the best for harmonisation purposes, although it was also mentioned that other methods for counting Varroa mites are currently used in monitoring schemes in Europe, as is the case of the soapy water or the sugar methods. The participants suggested that any proven method could be used, although it was clear that this would result in difficulties to compare the data (e.g. analysis on individual bees versus a few hundred bees). It was noted that it is important to highlight in the main section of the scientific opinion that the presence of brood on the timing of assessment should be reported, as well as previous treatments against *Varroa*, to allow a clear understanding of the test results and the relation with colony infestation levels.

With regard to the methods for testing for *P. larvae* and *M. plutonius* the participants were asked if the use of lateral flow kits would be a good option for testing for these agents in the field. The participants mentioned that these tests are only used in some MSs for an initial screening when larvae showing typical clinical signs are present in the hive. This initial screening is then followed by laboratory confirmation. For the purpose of large scale monitoring, where both healthy and diseased larvae may be tested, these tests may not be a good option due to the possibility of false negatives, which should be avoided, considering the severity of AFB disease. More information on this test

system is required, for instance on its capacity to detect different genotypes of the bacteria. Apart from the lateral flow kits, the best matrix to be sampled in a surveillance context was debated. According to the experience of various participants, the matrix used could be adult bees, honey samples or debris for testing for AFB. In most cases, a PCR test would provide more accurate results.

Regarding Nosema testing, the participants highlighted the importance of distinguishing between *Nosema apis* and *Nosema ceranae*, due to the different virulence of the species. It was mentioned that *Nosema* disease dynamics are not yet fully understood, and that environmental and genetic factors may play a role in *Nosema* prevalence. The participants were asked if there are data relating the number of *Nosema* spores to the presence of clinical signs. It was mentioned that in Greece this pathogen is considered the cause of death if more than 1.5 million of *N. ceranae* spores/bee are detected in a dead colony.

Comments related to the interaction between indicators and/or factors (e.g. relation between diseased colonies and overwintering capacity) were not discussed in the workshop, because the assessment of the interaction between indicators is beyond the scope of this mandate. The opinion section addressing TOR4 will give some guidance on how to identify associations between indicators and/or factors that affect bee health.

4. Discussion of external drivers

4.1. Resource Providing Unit

During this session, participants provided general comments regarding the definition of a 'resource providing unit' (RPU). It was agreed to define the average foraging distance as a proxy for habitat quality and resource availability, specifying that this also depends on the colony size. The experts suggested amending the RPU definition of foraging distance covered by the forager bees, i.e. average of 3 km foraging distance and 10 km for maximum distance from the hive.

With regard to the factor 'land cover', the participants pointed out that the classification presented did not reflect the resource availability within the RPU. Although the land cover description for a study performed at EU level could be limited to the CORINE Land Cover, it was suggested to use a better spatial and temporal resolution of the land cover classification, reflecting the type of forage (i.e. crops availability) and the forage seasonality (i.e. flower blooming period). Moreover, the participants stressed that the agronomic practices are linked directly to the land use, thus they should be addressed at crop-temporal scale when assessing the factor 'land cover'. In order to characterize 'land cover', it was suggested to take into account methods such as GIS[3] tools, waggle dance, palynological and pesticides residues analysis on crops. Some participants also mentioned that detailed land use information in the RPU does not always reflect the resources that are explored by the forager bees.

When discussing the group of factors 'Contamination of Environmental Matrices', participants stated that a clear definition of 'contaminant' is needed. Electromagnetic fields could be considered under the factor 'other contaminations'. On the other hand, it was agreed that heavy metals are not relevant for honeybee health. It was suggested to reword the factor 'bee feed' by 'bee forage' in order to differentiate it from supplementary feeding (which is covered in the Beekeeping Management Practices section). Concerning the factor 'contamination in puddle water', some participants pointed out that the scoring (related to the technical feasibility and the priority in field survey) should be revised since this was assessed in previous studies within the EU and was reported to be an important source of contamination. Another participant pointed out that the assessment of puddle water and its potential importance may be problematic due to difficulties to generate concrete evidence of a bee colony link to specific puddles, and to the ephemeral nature of puddles.

Finally, general comments were provided. In particular, it was highlighted that digital tools (e.g. mobile applications for mapping RPU) should be further developed and could contribute to harmonising data collection across EU. Existing datasets on pollen availability and high resolution land use/land cover maps should be further explored (e.g. UK Land Cover Map series[4]). Regarding the

[3] Geographic information system

[4] http://digimap.edina.ac.uk/webhelp/environment/data_information/landcover_data.htm, last accessed 10 June 2016

actors involved the data collection to measure the RPU variables, the participants agreed the activity should be carried out by beekeepers, national institutions, beekeepers associations and/or bee inspectors.

4.2. Environmental drivers

During this breakout session, the 'environmental drivers' (ED) defined as abiotic factors belonging to three categories (soil, weather and climate) were discussed. The focus was on the interactions between EDs and indicators such as colony attributes and beekeeping management practices (BMP). The WG clearly remarked the importance of this aspect and confirmed that analysis of associations between EDs and other indicators will be described in TOR4. Several examples were mentioned on how it could be possible to link EDs to land cover, topography and temperature to predict species likelihood of occurrence, proxies for pollination services. For instance, examples of spatially-explicit models assessing the potential of pollinator service across Europe were presented, making use of recent and ongoing projects carried out by the UK Insect Pollinators Initiative (https://wiki.ceh.ac.uk/display/ukipi/Home) and the European Commission Joint Research in Ispra, Italy. The discussion highlighted that to assess pollination services, fine spatial and temporal resolution of ED information are extremely important (i.e. climate / weather, land use / land cover and land management), as well as high quality information on 'beekeeper type', 'beekeeper experience' and 'quantity of colonies' per beekeeper. The importance of nectar availability in the RPU (which is affected by EDs such as temperature and relative humidity) was also identified as crucial for assessing pollination services. The interest to use predictive models was also highlighted for anticipating possible climatic scenarios on pollination services or to model predators and pests spread across Europe.

The second part of the session mainly focused on data availability across Europe. Participants pointed out that gaps currently exist on the principal key drivers (e.g. pesticide residues). Bee health and pollination services are highly dependent on daily temporal and climatic variations as well as specific microclimatic conditions and exceptional climatic events. Therefore, high quality data that aim to capture and correctly describe those variations are of major importance when developing predictive models. It was stated that data are scattered or protected by confidentiality and there is lack of data harmonisation and representativeness across Europe. The need of interaction between Agricultural Research Centres and Beekeeper Associations was stressed as it is required to share available data.

4.3. Beekeeping Management Practices

The mapping of the BMP factors and the main groups of factors 'beekeeper characteristics', 'colony management' and 'apiary characteristic and management' were presented.

With regard to 'beekeeper characteristics', the participants pointed out that the 'migration activity' and 'production system' of the beekeeper (organic/conventional) should also be considered as variables characterising the beekeeper category factor. The production system should be further detailed. The participants also considered that the hive rental for crop pollination is an increasing activity in the EU MSs. The participants confirmed that the number of hives managed is a relevant variable, but that there is a large variation across MSs. Therefore, linking it with RPU factors was considered essential when assessing the 'beekeeper category'. The participants stressed that the income of the beekeeper activity is a difficult variable to estimate, especially because it depends on the economic situation of each EU country. Therefore, stakeholders suggested defining this variable as "significant source of income (Yes/No)" and that its relation to the economical context of the country should be considered. When considering the variable 'average production per colony in the previous years', it was suggested to take into account the previous 5 years. If the beekeeping activity was <5 years, then the whole duration of the beekeeping activity would be considered. For the 'beekeeper experience', the participants suggested to include the beekeeper gender and the ability to recover colonies as new variables. Beekeepers could recover colonies by buying new colonies/bee packages or through their technical ability/experience (e.g. queen breeding, good management practices), hence these details should be addressed. It was also stated that the trend of number of colonies managed over time should be monitored.

With regard to 'colony management', and in the context of 'introduction of comb foundation' practice the participants suggested to address specifically the *Paenibacillus larvae* contamination in combs made of used wax. Although the agent is already addressed in the section dealing with the colony

attribute “Disease” (by testing biological matrices such as worker bees or brood), from a beekeeping management practices perspective, it would be necessary to assess the disease prevention measures performed by the beekeeper. In addition, the participants highlighted that the veterinary medicines used for chemical control in the hive should be specified. The ‘physical control methods’ factor was discussed and it was suggested to provide a clear definition or to reword the factor (replace it with ‘mechanical control methods’). The ‘biotechnical control methods’ should be explained in the text to facilitate the understanding of these factors. In this context, participants suggested to take into account the integrated treatments (e.g. organised by beekeeping organisations) as a new variable, so the possible cross-contamination between different apiaries could be addressed. The priority of swarm control for field assessments was discussed and it was suggested to revise the priority of this factor to high. This suggestion was not included in the EFSA draft document considering the effort required to monitor the swarming activity at the colony level (in spring, approximately every 10 days a colony inspection is required).

The participants highlighted that beekeepers management practices play a central role to measure the health status of the honeybee colony and to achieve the production objectives in terms of colony outputs.

5. Discussion of colony outputs

5.1. Colony outputs - Provisioning and Regulating Services

Regarding the “Colony Outputs”, participants pointed out that a clear definition of “honeybee colony” is needed and capturing its timeframe is considered essential (e.g. if the beekeeper replaces the queen bee, the colony itself changes and this is not considered as colony loss).

With regard to provisioning services, the participants indicated the increasing importance in the EU of the “hive rental” activity for pollination of cultivated plant species. This activity is strictly linked to the Beekeeping Management Practices and it was commented that the colony health status should be checked prior to migration, in order to prevent the cross contamination of colonies with diseases/infections/infestations.

With regard to the regulating services, in particular to the pollination service, stakeholders emphasised the difficulty to estimate the pollination service in a specific RPU provided by a specific honeybee colony if several hives are in the area.

It was mentioned that pollen analysis in the field does not fully reflect the service provided by a honeybee colony (other pollinators are also providing the service). Measuring the pollen in the honey, although being more precise, it is still not good enough as pollen might be lost in flight. In addition, some crops are pollinated by honey bees but only low amounts of the respective crop’s pollen are found since nectar is predominantly collected (e.g. citrus). Therefore from a monitoring perspective, all apiaries within the Resource Providing Unit are a more pragmatic unit of measurement for pollination service; this is due to the high variability of services between colonies of different size, and to the lack of information at the spatial resolution of the RPU. Moreover, it was suggested to take into account other pollinator species within the Resource Providing Unit when assessing pollination services. Stakeholders agreed on considering the floral survey when assessing the “Resource Providing Unit” factors and on referring back to it in the modelling methods of Colony Outputs section. In the scientific opinion, the modelling description should be further expanded and the literature updated.

6. Conclusions and next steps

In the last plenary session of the workshop, the next steps were presented and the main outcomes of the workshop were formulated.

The detailed discussions during the breakout session proved to be very useful. Additional scientific evidence was provided, the feasibility of several methods was discussed as well as the needs and limitations regarding harmonisation on testing and reporting within the EU. The collected information will be considered by the WG when finalising the draft scientific opinion. In general, there was agreement on the mind maps although some modifications will be done based on the workshop

discussions: in particular, additional indicators and factors will be added and some scores will be reviewed. A pending question is how the selected indicators and factors will be combined when analysing the data. This was not discussed during the workshop as it is part of TOR4, which is more technical and mainly addressed by WG members with a background in statistics. Once information on indicators and factors identified in TORs 2-3 of the HEALTHY-B mandate will have been collected, statistical methods (TOR 4) should be applied to test their correlation and reveal significant interactions. This might result in the identification of a subset of key indicators and factors affecting the health status of a managed honeybee colony, as defined in TOR1 of the mandate. This would mean a reduction of the number of indicators and factors to be analysed in the future. Overall, this is an evolving process, which needs to be fine-tuned to reflect best available information (i.e. data, methods and knowledge). The importance of a field survey design was mentioned several times during the workshop and it will also be mentioned under TOR4. For clarification on the "field work" concept used in the EFSA scientific opinion, it was suggested to rephrase it with "primary data collection" as it would be more consistent with the aim of the document.

The framework generated by the HEALTHY-B mandate is perceived as very theoretical, and stakeholders expressed the need to understand how it can be practically implemented. EFSA explained that the HEALTHY-B framework will be used by the MUST-B WG to identify the indicators and factors that can be incorporated into a model that could be used for the risk assessment of pesticides in the context of multiple stressors. A procurement will be launched in 2016 to call for the creation of a consortium capable of developing such a mechanistic, exposure/effect and agent-based model. In a second step, data collection will be organised at representative sites across the EU to evaluate the performance of the model. A procurement call on this activity is foreseen early 2017. The HEALTHY-B opinion is foreseen to be in the public domain in October 2016 and will be used in the design of this data collection.

Stakeholders asked to clarify how the HEALTHY-B scientific opinion may be used in future, beyond EFSA's objectives. In essence, it provides guidance to further harmonise data collections in field conditions across the EU. It can be seen as a toolbox from which one could select what is needed and relevant according to the specific objectives of a given study. Further improvements of the 'tools' and gradual uptake by the groups interested in bee health would provide opportunities to compare studies and would facilitate the implementation of meta-analyses. A section will be included in the opinion to explain how the HEALTHY-B framework could be used by different stakeholder groups such as risk assessors, beekeepers, farmers and risk managers.

The participants were also informed on the EU scientific workshop on bee health and sustainable pollination that recently took place in EFSA (10 March 2016[5]). Within that workshop, a group of twenty-one representatives of the EC, EU-funded bee projects, US-EPA, EEA, EURL, OIE, JRC, EMA, IPI, EFSA and bee experts discussed and ranked research recommendations. The EC is considering the top five recommendations in relation to upcoming Horizon 2020 calls.

Finally, Louis Mahy (DG-RTD, EC) informed the workshop participants on the timing of 'bee'-calls. Two calls are expected to be open from 4 October 2016 with a first deadline on 14 February 2017. It is expected that information will be made available in May 2016.

[5] https://www.efsa.europa.eu/en/supporting/pub/1026e, last accessed on 10 June 2016

Abbreviations

AFB	American Foulbrood
BMP	Beekeeping management practice
Corine Land Cover	Coordination of Information on the Environment Land Cover
EC	European Commission
ED	Environmental driver
EEA	European Environmental Agency
EFB	European Foulbrood
EMA	European Medicines Agency
EU	European Union
EURL	European Reference Laboratory for Honeybee health
GIS	Geographic information system
HEALTHY-B	EFSA project on the Health Status of a Managed Honeybee Colony
IPI	Insect Pollinators Initiative
JRC	Joint Research Centre
MS	Member State
MUST-B	EFSA project on Multiple Stressors in Bees
OIE	World Organisation for Animal Health
RPU	Resource Providing Unit
TOR	Terms of Reference
US-EPA	United States Environmental Protection Agency
WG	Working group