SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 13 September 2016

doi: 10.2903/j.efsa.2016.4578

Assessing the health status of managed honeybee colonies (HEALTHY-B): a toolbox to facilitate harmonised data collection

EFSA Panel on Animal Health and Welfare (AHAW)

Abstract

Tools are provided to assess the health status of managed honeybee colonies by facilitating further harmonisation of data collection and reporting, design of field surveys across the European Union (EU) and analysis of data on bee health. The toolbox is based on characteristics of a healthy managed honeybee colony: an adequate size, demographic structure and behaviour; an adequate production of bee products (both in relation to the annual life cycle of the colony and the geographical location); and provision of pollination services. The attributes 'queen presence and performance', 'demography of the colony', 'in-hive products' and 'disease, infection and infestation' could be directly measured in field conditions across the EU, whereas 'behaviour and physiology' is mainly assessed through experimental studies. Analysing the resource providing unit, in particular land cover/use, of a honeybee colony is very important when assessing its health status, but tools are currently lacking that could be used at apiary level in field surveys across the EU. Data on 'beekeeping management practices' and 'environmental drivers' can be collected via questionnaires and available databases, respectively. The capacity to provide pollination services is regarded as an indication of a healthy colony, but it is assessed only in relation to the provision of honey because technical limitations hamper the assessment of pollination as regulating service (e.g. to pollinate wild plants) in field surveys across the EU. Integrating multiple attributes of honeybee health, for instance, via a Health Status Index, is required to support a holistic assessment. Examples are provided on how the toolbox could be used by different stakeholders. Continued interaction between the Member State organisations, the EU Reference Laboratory and EFSA is required to further validate methods and facilitate the efficient use of precise and accurate bee health data that are collected by many initiatives throughout the EU.

Keywords: Honeybee, colony, health, field, attribute, indicator, toolbox

Requestor: EFSA

Question number: EFSA-Q-2015-00047

Correspondence: ALPHA@efsa.europa.eu

Panel on Animal Health and Welfare (AHAW) members: Miguel Angel Miranda, Dominique Bicout, Anette Botner, Andrew Butterworth, Paolo Calistri, Klaus Depner, Sandra Edwards, Bruno Garin-Bastuji, Margaret Good, Christian Gortazar Schmidt, Virginie Michel, Simon More, Søren Saxmose Nielsen, Mohan Raj, Lisa Sihvonen, Hans Spoolder, Jan Arend Stegeman, Hans H. Thulke, Antonio Velarde, Preben Willeberg, Christoph Winckler

Acknowledgements: The AHAW Panel wishes to thank the HEALTHY-B working group members Gérard Arnold, Thomas David Breeze, Howard Browman, Magali Chabert, Margaret Couvillon, Gianni Gilioli, Pascal Hendrikx, Daniel Oberski, Chiara Polce, Marie-Pierre Rivière, Ullrika Sahlin, Simone Tosi; the EFSA Panel on Plant Health (PLH); Claude Bragard, David Caffier, Thierry Candresse, Elisavet Chatzivassiliou, Katharina Dehnen-Schmutz, Gianni Gilioli, Jean-Claude Gregoire, Josep Anton Jaques Miret, Michael Jeger, Alan MacLeod, Maria Navajas Navarro, Bjoern Niere, Stephen Parnell, Roel Potting, Trond Rafoss, Vittorio Rossi, Gregor Urek, Ariena Van Bruggen, Wopke Van Der Werf, Jonathan West and Stephan Winter; the hearing experts: Ann Alix, Koos Biesmeijer, Etienne Bruneau, Martin Dermine, François Diaz, Thierry Grollier, Walter Haefeker, Klemens Krieger, Hans Mattaar, Simone Tosi, Romée Van der Zee, Geoffrey Williams for the preparatory work on this scientific opinion and EFSA staff members: Domenica Auteri, Edoardo Carnesecchi, Gilles Guillot, Eliana Lima, Agnes Rortais, Giorgio Sperandio, Franz Streissl, Frank Verdonck, Stefania Volani and Sybren Vos for the support provided to this scientific opinion.

Suggested citation: EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2016. Scientific opinion on assessing the health status of managed honeybee colonies (HEALTHY-B): a toolbox to facilitate harmonised data collection. EFSA Journal 2016;14(10):4578, 241 pp. doi:10.2903/j.efsa.2016.4578

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

The European Food Safety Authority (EFSA) asked the Panel on Animal Health and Welfare (AHAW) to generate a toolbox to facilitate data collection to support assessing the health status of managed honeybee colonies. The mandate requested identification of the main characteristics of a healthy honeybee colony, which data can be collected in field surveys across the European Union (EU), how to measure and report variables in a harmonised manner and how data on bee health could be analysed.

This scientific opinion aimed to provide an overview of tools that could be used in the assessment of bee health, which is an element of a larger process to achieve EFSA's objective to evolve towards an integrated risk assessment approach for bees. Any analysis of bee health is recommended to start by defining the goals and purpose of the analysis, and then work backward to the analysis approach and data collection effort needed to achieve those goals. In this opinion, the objective of a bee health assessment is not specified in detail to enable any organisation involved in such activities to select tools from the generated HEALTHY-B toolbox according to their specific objectives. For instance, it is recommended to use the tools that are relevant across the EU (e.g. for *Varroa* quantification) and select some additional tools that are specific for a given area in the EU (e.g. for small hive beetle detection). The long-term objective is to improve test method validation, data collection, reporting and analysis across the EU, which will facilitate risk assessment on bee health by the national and the European risk assessment bodies. This guidance, in fact, provides a set of tools that are or could be harmonised, validated and suitable for data analysis and comparisons, without imposing too rigid a framework. More than one validated protocol might be used to measure an indicator or factor if the collected data can be merged in the analysis phase. Interaction between many stakeholders is required to bring test method validation and data collections forward. Beekeepers are an important target audience for this paper because they play a major role in collecting data in the field and their subsequent submission to the scientific community. In-depth training of beekeepers and bee inspectors is key as the quality of the analysis is dependent on the accuracy and precision of the collected data.

Bee health is considered in this opinion in its broader sense, meaning that it is dependent on several high-level characteristics that describe bee health in a holistic manner at the colony level. A colony of managed honeybees was defined as an *Apis mellifera* bee population kept by a beekeeper with the presence of a given queen. Replacement of the queen by a natural process or by a beekeeper is considered to result in a new colony because it changes the genetics of the population. Based on a scoping of the scientific literature and subsequent discussion by working group (WG) members and hearing experts representing different stakeholders, it was concluded that the characteristics of a healthy managed honeybee colony are: an adequate size, demographic structure and behaviour in relation to the annual life cycle of the colony and the geographical location; an adequate production of bee products in relation to the annual life cycle of the colony and the geographical location; and provision of pollination services. The identification of these characteristics served as the basis for the development of a hierarchical approach. The highest hierarchical level consists of three overarching concepts that reflect the multidimensional characteristics of: (i) a managed honeybee colony; (ii) its habitat and management; and (iii) its productivity from the perspective of human interest, referred to as 'colony attributes', 'external drivers' and 'colony outputs', respectively. The three overarching 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).

An overview of the identified indicators and factors from field surveys was made and was used as a basis to generate summaries presented in the form of mind maps on indicators and factors for colony attributes, external drivers or colony outputs. The indicators and factors were scored (high or low) for their relevance to the health status of a managed honeybee colony or the relevance to understanding the context of a managed honeybee colony, respectively; for their technical feasibility in the context of field surveys; and priority for inclusion in field surveys across the EU. The indicators and factors with an H-HH score (H-HH meaning High relevance, High technical feasibility and High priority) were further scrutinised to identify the most relevant variable(s) and method(s) to quantify them. The opinion provides detailed information on the available test methods, suggesting which of these are most suitable for implementation in field surveys across the EU and specifying the most appropriate reporting units. The identification, scoring, measurement and reporting of indicators and factors have been discussed by scientists, beekeepers, risk managers and representatives of other stakeholder groups during a workshop to collect scientific evidence that was not yet identified by the WG.

Indicators describing the colony attributes 'queen presence and performance', 'demography of the colony', 'in-hive products' (including their contaminants) and 'disease, infection and infestation' can be

measured in field surveys across the EU although efforts are required to implement these in a harmonised manner. In particular, the generation of detailed protocols and the validation of many test methods are necessary. The colony attribute 'behaviour and physiology' is difficult to measure in field surveys and the available technology is currently restricted to experimental studies, except for the detection of explicit atypical behaviour. External drivers of honeybee health consist of factors related to the resource providing unit (RPU; environmental components around the hive including contaminants), environmental drivers (weather and climate) and beekeeping management practices. Analysing the RPU, in particular land cover/use, of a honeybee colony is very important when assessing the health status of a colony, but it currently lacks tools that could be used at the apiary level in field surveys across the EU. Data on 'beekeeping management practices' and 'environmental drivers' can be collected via questionnaires and available databases, respectively. Some existing databases containing relevant (and validated) data to assess bee health are listed, but efforts are required to further increase the public accessibility of these data. For the attribute 'colony outputs', provisioning services can be analysed mainly for harvested honey, whereas technical limitations hamper the assessment of regulating services (such as the pollination of wild plants) in field surveys across the EU. Moreover, there is a significant lack of information that quantitatively links pollination services to colony health; however, using modelling approaches it is possible to link pollination services with other colony attributes and external drivers. In a multifactorial risk assessment of honeybees, the impacts on pollination services should be estimated.

Overviews of indicators and factors related to bee health are provided (Chapter 3) and a selection has been made of those that could be included in a field survey across the EU (Chapter 3 and summary in Chapter 4). It is clear that the design of detailed, harmonised protocols and the validation of several tools together with adequate training are required, before multiannual collection of data and their analysis would be possible in a harmonised manner at the EU level, in particular if accurate and precise quantitative data are required. The subsequent chapters provide guidance on key elements to consider when designing a field survey (Chapter 5) and analysis of bee health data (Chapter 6).

The key elements to consider in the stage of designing a field survey are: (i) carefully designing and implementing each aspect of the survey; (ii) ensuring that ample resources are dedicated to this aspect of the project; and (iii) ensuring in advance of any data collection that the design choices allow for the desired analyses. Reference is made to several guidance documents that are available in the public domain and that are recommended to consult whenever more detailed information is required.

As specified above, there are no *a priori* key variables representing unequivocally the health status of a honeybee colony because this is influenced by many variables and their interactions. Therefore, multiple indicators should be considered jointly in an analysis of bee health. Chapter 6 gives a short overview of sensible approaches to integrate data on bee health to provide an overall outcome. There are many suitable approaches available and four are described: (i) multivariate analysis, (ii) expert-driven classification, (iii) causal modelling and (iv) process-based modelling. These approaches are related to each other and can overlap. The first two approaches represent alternative ways to define a Health Status Index (HSI) in a way that the assessment is based on more than one indicator, whereas the third and fourth approaches describe ways to link factors to health and to model changes in health.

The information provided in this opinion is a basis to facilitate harmonised data collection across the EU, without predefining a specific objective. The latter was a decision made to allow use of the HEALTHY-B toolbox when bee health is assessed in relation to various objectives and analysis goals. However, not defining a specific objective and analysis goal made it difficult for the authors to be very precise in the selection of indicators, factors, methods and the formulation of recommendations. As a consequence, further actions will be required to translate the information provided in this document into a study protocol that can be implemented in practice and that is in line with a clearly defined objective. Chapter 7 provides some examples on the possible use of the HEALTHY-B toolbox by different stakeholder groups: monitoring and comparison of honeybee health over time and across geographical space, identification of possible factors and indicators that can predict changes in the health status of a managed honeybee colony, pesticide risk assessment in the context of multiple stressors.

Intensive data collections at a few places across Europe are required to develop an HSI and risk assessment models. In addition, an epidemiological study involving many apiaries across the EU is necessary to provide complementary information to analyse the relative importance of different stressors, which could then be incorporated in the HSI and/or used by relevant models. The required

precision and accuracy of the data will be important in the test method selection and defining the role of beekeepers and bee inspectors in the data collection.

The HEALTHY-B toolbox is currently used in EFSA's Multiple Stressors in Bees (MUST-B) project, which aims to develop a predictive model that could be used as a tool by risk assessors and managers to determine risks of pesticides in honeybee colonies under different scenarios of exposure to multiple stressors. Several stakeholders could benefit by applying the toolbox, for instance via harmonisation of data collection/reporting, more efficient use of data collected across the EU, beekeeper involvement in bee health assessments, and a basis on which to develop online tools that are mutually beneficial to beekeepers, scientists and risk assessors/managers.

Table of contents

Abstract 1
Summary 3
1. Introduction 8
1.1. Background and Terms of Reference as provided by the requestor 8
1.2. Interpretation of the Terms of Reference 9
1.3. Target audience 10
2. Data and methodologies 11
2.1. Hierarchical approach 11
2.1.1. Identification of the overarching concepts of a managed healthy honeybee colony 11
2.1.2. Identification of indicators and factors 12
2.1.3. Identification of variables and methods 12
2.2. Procedure for selection of indicators and factors 12
2.2.1. Procedure and scoring system used 12
2.2.2. Data collection in field surveys 14
2.3. Workshop 15
3. Assessment 16
3.1. Identification of the colony attributes, external drivers and colony outputs (TOR1) 16
3.1.1. Characteristics of a managed healthy honeybee colony 16
3.1.2. Colony attributes 16
3.1.3. External drivers 16
3.1.4. Colony outputs 17
3.2. Colony attributes reflecting the health status of a managed honeybee colony (TOR2–3) 17
3.2.1. Queen presence and performance 17
3.2.1.1. Identification of indicators related to queen presence and performance (TOR2) 17
3.2.1.2. Methods and tools to measure indicators related to queen presence and performance (TOR3) 18
3.2.2. Demography of the colony 20
3.2.2.1. Identification of indicators related to demography of the colony (TOR2) 20
3.2.2.2. Methods and tools to measure indicators related to demography of the colony (TOR3) 22
3.2.3. In-hive products 24
3.2.3.1. Identification of indicators related to in-hive products (TOR2) 24
3.2.3.2. Methods and tools to measure indicators related to the in-hive products (TOR3) 27
3.2.4. Behaviour and physiology of the bees 29
3.2.4.1. Identification of indicators related to behaviour and physiology of the bees (TOR2) 29
3.2.4.2. Methods and tools to measure indicators related to behaviour of the bees (TOR3) 33
3.2.5. Disease, infection and infestation 34
3.2.5.1. Identification of indicators and methods related to disease (TOR2 and TOR3) 34
3.2.5.2. Identification of indicators related to infection or infestation (TOR2) 35
3.2.5.3. Methods and tools to measure indicators related to infection or infestation (TOR3) 38
3.3. External drivers affecting the health status of a managed honeybee colony (TOR2-3) 40
3.3.1. Resource providing unit (TOR2) 40
3.3.1.1. Relevance of the RPU factors to the bee health status of a colony 41
3.3.1.2. Technical feasibility and priority to include RPU factors in field surveys 42
3.3.1.3. Methods and tools to measure factors related to RPU (TOR3) 43
3.3.2. Environmental drivers (TOR2) 45
3.3.2.1. Relevance of the environmental drivers to the bee health status of a colony 46
3.3.2.2. Technical feasibility and priority to include factors on environmental drivers in field surveys 46
3.3.2.3. Methods and tools to measure factors related to environmental drivers (TOR3) 46
3.3.3. Beekeeping management practices 47
3.3.3.1. Relevance of the beekeeping management practices to the bee health status of a colony 48
3.3.3.2. Technical feasibility and priority to include factors on beekeeping management practices in field surveys 49
3.3.3.3. Methods and tools to measure factors related to beekeeping management practices (TOR3) 50
3.4. Colony outputs (TOR2-3) 53
3.4.1. Relevance of colony outputs to the bee health status of a colony 53
3.4.2. Technical feasibility and priority to include colony output indicators relevant to bee health status in field surveys 54
3.4.3. Methods and tools to measure factors related to colony outputs 54
4. Field data collection: which indicators and factors to include across the EU 55
5. Field data collection: considerations during survey design (TOR4) 57
5.1. Data validation 58

5.2. Data management and analysis system 59
6. Field data collection: options for data analysis (TOR4) 59
6.1. Background 59
6.2. Analysis output: goals of a bee health analysis 60
6.2.1. Descriptive 60
6.2.2. Explanatory (sometimes called 'diagnostic') 60
6.2.3. Predictive 61
6.2.4. Prescriptive 61
6.3. Analysis production: approaches to modelling bee health 62
7. Use of the toolbox for different objectives and by different stakeholder groups 63
7.1 Example 1 – Monitoring and comparison of honeybee health over time and across geographical space 64
7.1.1. Background and objective 64
7.1.2. What is an HSI for managed honeybee? 64
7.1.3. How does the HEALTHY–B toolbox help to generate an HSI? 64
7.1.4. How could the HSI be used? 65
7.2. Example 2 – Identification of key predictors of change in honeybee health 66
7.2.1. Background and objective 66
7.2.2. How does the HEALTHY–B toolbox help to identify key health (status) predictors? 66
7.2.3. How could prediction of changes in bee health status be used? 66
7.3. Example 3 – Pesticide risk assessment on honeybee health in the context of multiple stressors 67
7.3.1. Background and objective 67
7.3.2. How does the HEALTHY–B toolbox help to introduce a holistic perspective into pesticide risk assessment? 68
7.3.3. How could a holistic pesticide risk assessment be used? 68
8. Conclusions and recommendations 69
8.1. Overarching TORs 1-4 69
8.1.1. Overarching conclusions 69
8.1.2. Overarching recommendations 70
8.2. TOR1: Identification of the colony attributes, external drivers and colony outputs 70
8.2.1. TOR1-specific conclusions 70
8.3. TOR2: Identification of indicators and factors relevant to measuring colony attributes, external drivers and colony outputs. TOR3: Methods and tools to measure indicators and factors relevant to measuring colony attributes, external drivers and colony outputs 71
8.3.1. Specific conclusions and recommendations on 'colony attributes' 71
8.3.2. Specific conclusions and recommendations on 'external drivers' 72
8.3.3. Specific conclusions on 'colony outputs' 72
8.4. TOR4: Propose a methodological approach to allow robust and harmonised measurement and comparison of regional bee health status 73
8.4.1. TOR4-specific conclusions 73
References 73
Glossary 93
Abbreviations 95
Appendix A – Examples of European studies monitoring bee health 96
Appendix B – Categorisation of identified indicators and factors 97
Appendix C – Measurement of selected indicators and factors 165
Appendix D – Clinical signs of disease 215
Appendix E – Contaminants in bee products 219
Appendix F – Worker behaviour catalogue 222
Appendix G – Protocol for data collection by the beekeeper on indicators scored as H–HH 223
Appendix H – Analysis of bee health 225

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

The way that stressors (mainly biological, chemical and environmental) affect honeybees (*Apis mellifera*) and contribute to 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 simultaneously and the effects of interactions between them.

In 2008, the European Food Safety Authority (EFSA) conducted a survey of existing bee surveillance systems in the European Union (EU; EFSA, 2008). Subsequently, the European Commission established an EU Reference Laboratory (EURL) for honeybee health[1] and funded an EU-wide monitoring programme on honeybee mortality events and the prevalence of specific bee pathogens in Europe (EPILOBEE[2]). However, given the large data set, high number of variables that are not yet fully analysed, and the absence of data on the monitoring of other bee stressors (i.e. chemical and environmental factors), the results from EPILOBEE must be considered preliminary.

EFSA seeks to develop, by 2018–2019, an integrated risk assessment approach for bees taking into account the multifactorial aspects of honeybee colony losses and weakening via the Multiple Stressors in Bees (MUST-B) project. In the present mandate, EFSA seeks to define: (i) what is meant by a 'healthy honeybee colony' and (ii) how can the health status of a honeybee colony be assessed in a robust and harmonised manner. The answers to these questions will provide guidance for designing studies that aim at systematically collecting data and analysing the health status of honeybee colonies in their natural environment at scales ranging from local through regional to international. Considered in a holistic sense, 'health' encompasses not only to the absence of pathogens and/or pests, but also, for instance, the capacity of the colony to produce honey and provide pollination services.

Information is already available on colony attributes that influence and/or determine the health status of a honeybee colony, as well as approaches and methodologies that assess honeybee health status. However, there is a need for a harmonised framework defining the indicators that should be measured when assessing the health status of a honeybee colony in large field surveys, which are agreed upon (and practical to implement) by stakeholders and feasible when applied at regional, national or international levels. This would result in more harmonised data collections in field surveys and hence facilitate meta-analysis and the inclusion of data in risk assessments. This framework should include indicators to measure the effects of the main biological, chemical and environmental stressors that affect the health status of a honeybee colony. In particular, the early signs of a deterioration in health need to be established. Harmonised frameworks have been developed for other multifactorial systems, such as the generation of an approach to assess animal welfare (EFSA Panel on Animal Health and Welfare, 2012; Welfare Quality Project[3]) and the environmental risk assessment of plant pests (EFSA Panel on Plant Health, 2011). It may be possible to apply elements of these methodologies – with appropriate modifications – to assess the health status of a honeybee colony. Although this mandate does not primarily aim to provide practical guidance to beekeepers on how to perform regular health checks of honeybee colonies, the framework could be used to assess the health status of one or a small number of colonies (e.g. within one apiary).

Once a framework is established, an inventory of available validated methods/tools that could be used to assess the health status of a honeybee colony in large-scale field surveys will be developed. This inventory should seek to identify gaps in our capacity to measure the health status of a large number of bee colonies in a relatively short time and hence recommend where method development and/or validation are required. Further, there is a need to provide guidance on how the data obtained from a survey could be analysed to ensure that data are collected appropriately, to allow for a harmonised interpretation across different ecosystems and to ensure applicability for future risk assessments. A colony of honeybees can cope with more stress than an individual honeybee, and this capacity might change seasonally and in relation to environmental conditions (to take into account regional differences across the EU Member States).

The output of this mandate is intended for use in two subsequent activities of the MUST-B project: (i) the design of protocols and field methods, and the calibration of tools, to allow robust and

[1] Commission Regulation (EU) No 87/2011.
[2] http://ec.europa.eu/food/animals/live_animals/bees/study_on_mortality/index_en.htm
[3] http://www.welfarequality.net/everyone/26559/7/0/22

harmonised assessment of honeybee colony health status; and (ii) the design and completion of a multifactorial honeybee colony field survey.

Terms of Reference:

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.

1.2. Interpretation of the Terms of Reference

Bee health is considered in its broader sense, meaning that it is dependent on several high-level characteristics describing bee health in a holistic manner at the colony level. The characteristics that should be taken into account when assessing the health status of a managed honeybee colony are defined in Terms of Reference (TOR) 1. These are the basis of a hierarchical approach that has been developed. The highest hierarchical level consists of three overarching concepts that reflect the multidimensional characteristics of: (i) a managed honeybee colony; (ii) its habitat and management; and (iii) its productivity from the perspective of human interest, referred to as 'colony attributes', 'external drivers' and 'colony outputs', respectively (Table 1). The three overarching 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 indicators and factors are considered to reflect the overarching concepts and can be derived by measuring one or more variables. For instance, 'queen potential fecundity' is an indicator describing the attribute 'queen presence and performance'. This indicator could be informed by measuring one of the following variables: viable egg-laying by the queen, rate of drones being laid, number of new queen cells per swarming event, and mating success (number of patrilines). TOR2 describes the biological relevance of indicators and factors regarding the health status of a managed honeybee colony. A ranking is presented for technical feasibility and priority for inclusion of an indicator or factor in field surveys that could be implemented across the EU.

Each indicator or factor can be described by one or more 'variables', which are quantified using a specific 'method'. TOR3 assesses the fitness for purpose and availability of methods to estimate the colony health status and that could be implemented in most Member States. However, it is clear that the generation of detailed protocols and the validation of many test methods are necessary before they can be implemented across the EU in a harmonised manner. Regarding data acquisition and analysis, the outputs of TOR2 and TOR3 should facilitate a comparison of data on the health status of managed honeybee colonies from different European regions. They should also assist the development of a harmonised data model, the merging of data sets and implementation of meta-analysis at the national and European level.

TORs 1–3 describes the current understanding of indicators and factors related to bee health, whereas TOR4 looks into the future and provides guidance on what to do when a field survey is planned. References are provided to documents giving guidance on the design of data collections. It also provides guidance to design the analysis and field data collection with respect to assessing the health status of managed honeybee colonies. This part of the scientific opinion describes that first the objective of a field survey should be defined (expected output), then the method(s) for data analysis should be selected and finally the collection of data should be designed and performed. Different types of outputs are presented and a description is provided of the main characteristics of some methods that might be relevant to analysis of the health status of honeybee colonies. It is intended to give an overview of some existing methods, explaining how they could be used and which important aspects have to be considered when designing a data collection.

Table 1: Hierarchical approach – levels of assessment and definitions

LEVEL 1	**External drivers**	**Colony attributes**	**Colony outputs**
Overarching concepts	Multidimensional characteristics of the colony habitat and management. Can only be assessed indirectly	Multidimensional characteristics that are an integral part of a health status of a managed honeybee colony. Can only be assessed indirectly	Multidimensional characteristics expressing the productivity of a managed honeybee colony from the perspective of human interest. Can be assessed both directly both indirectly
LEVEL 2	**Factors**	**Indicators**	**Indicators**
Abiotic or biotic components	A set of factors is used to assess the external drivers	A set of indicators is used to assess the colony attribute	A set of indicators is used to assess the colony outputs
LEVEL 3	**Variables**		
	Measurable quantities identified for each indicator and factor. One or more variables are used to estimate each indicator or factor		
LEVEL 4	**Methods**		
	Practical procedure to quantify the variable. One or more methods are available to estimate the same variable		

1.3. Target audience

Understanding the effects of indicators and factors on bee health requires information from several geographical areas, preferably collected at the same time. Collecting and comparing data between areas is a very complex task due to the heterogeneity of the European apicultural sector across the EU (Chauzat et al., 2013; Deloitte, 2013), let alone the environmental heterogeneity. This scientific opinion aims to provide an overview of tools that could be used for the assessment of bee health, which is an element of a larger process to achieve EFSA's objective of evolving towards an integrated risk assessment approach for bees. Efforts to improve test method validation, data collection, reporting and analysis across the EU will facilitate risk assessment on bee health by national and European risk assessment bodies. This guidance, in fact, provides a set of tools that are harmonised and would allow data analysis and comparisons, without imposing a too rigid framework. More than one protocol might be used to measure an indicator or factor if these generate data that can be merged in the analysis phase. One could use tools that are relevant across the EU (e.g. for *Varroa* quantification) and select some additional tools that are specific for a given area in the EU (e.g. for small hive beetle detection). Beekeepers play a major role in collecting data in the field and are, therefore, an important target audience for this paper, in particular, guiding them on how data could be submitted to the scientific community. Chapters 5 and 6 cover key elements on the design of a field survey and provide some examples on how data on bee health could be analysed, as an introduction to anybody planning a field survey and to indicate the requirement of a multidisciplinary team when assessing bee health in a holistic manner. Furthermore, as illustrated in Figure 1, connecting new and existing open databases with information that is reliable and relevant to bee health would increase the openness and transparency of risk assessment and would facilitate using the data for other purposes, such as management and decision-making processes by beekeepers and/or officials at a regional, national or international level.

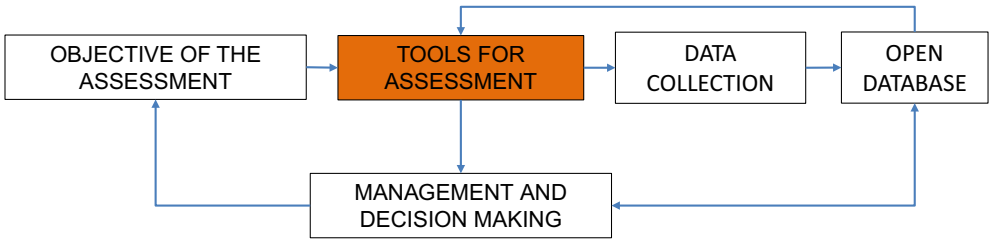


Figure 1: The objective of this scientific opinion is to provide an overview of tools that could be used for the assessment of bee health, which is part of a larger process to achieve EFSA's objective to evolve towards an integrated risk assessment approach for bees to further facilitate science-based management and decision making

Definition of the objective will help identify which tools are selected for the assessment. In this scientific opinion, the aim is to facilitate harmonised data collection to assess the health status of a managed honeybee colony from a holistic perspective and following a framework that can be applied across the EU (see Section 2.2.2). The identified methods/tools will allow further harmonisation of data collections on bee health within Europe and should facilitate more robust data analysis in a context of simultaneous exposure to different stressors and continuously changing environmental conditions. Further actions will be required to translate the information provided in this document into a precise study protocol and to validate test methods that can be implemented in practice and are in line with a clearly defined objective.

The toolbox described in this scientific opinion can be consulted by everybody involved in measuring, reporting and analysing bee health in the EU. It will also be used by the EFSA MUST-B working group (WG) to: (i) select indicators and factors that could be included in a model assessing the effect of pesticides on bee health and (ii) design a field survey to collect data to inform the parameters of the model. In addition, the outcome of this opinion could also be used by risk assessors and scientists as a basis for epidemiological studies to identify associations between indicators and factors, particularly when data from a large area are required (e.g. to investigate the correlations between *Nosema* spp. and mortality, observed in the south of Europe, but not in the north). This paper also targets people beyond the scientific community. It is not a practical guide on how to keep a honeybee colony healthy. However, it explains how beekeepers could provide data on their colonies to facilitate scientific analysis. In fact, the provided guidance is another step towards involving beekeepers in science and risk assessment. It is clear that further actions are required to achieve its implementation in a practical and efficient manner. Several methods described in the opinion are time consuming, require in-depth training and/or efforts to assure standardised implementation of the protocol, which may limit the persons collecting the data to specifically trained beekeepers and/or bee inspectors.

Better knowledge on the drivers affecting bee health will result in improved assistance to beekeepers and farmers, hence optimising their outputs, such as honey production and/or pollination services. Therefore, the output of this scientific opinion is assumed to be useful for different stakeholder groups, each having different objectives. Chapters 3, 4, 5 and 6 describe the tools in the toolbox, whereas Chapter 7 explains how the toolbox could be used by different stakeholder groups. Beekeepers and bee inspectors are the key actors in collecting and reporting the data, whereas several other groups have interest in obtaining good quality data. Models, for instance, need as precise and accurate data as possible to generate outputs reflecting the field situation.

This scientific opinion provides arguments on the current scientific knowledge to assess the health status of managed honeybee colonies in Europe.[4] Only managed honeybees (*Apis mellifera*) are considered here, because most knowledge and techniques are available for this bee species. It is believed that some of the tools in the 'toolbox' are applicable or adjustable to also suit other bee species. Expanding the analysis to other bee species would be a useful addition, as bee diversity is very important, for instance, for the delivery of pollination services (EFSA, 2013, 2014).

2. Data and methodologies

2.1. Hierarchical approach

2.1.1. Identification of the overarching concepts of a managed healthy honeybee colony

Similarly to the conceptual framework developed to assess the welfare status of farmed animals based on the five freedoms (EFSA, 2012b[5]), the Panel on Animal Health and Welfare (AHAW) developed in this scientific opinion a framework to assess the health status of a managed honeybee colony based on a few overarching concepts. A scoping[6] of the scientific literature has been carried out in Web of Science using the following search strings:

[4] This scientific opinion does not aim to review the current knowledge on bee health, or to provide guidance to the scientific community on future research activities.

[5] http://www.welfarequality.net/everyone/26559/7/0/22

[6] This approach was considered to be an efficient manner to identify the overarching concepts, indicators and factors. Detailed literature reviews were not performed since it is not the aim of this scientific opinion to provide an overview of all the available scientific evidence for each indicator or factor. A workshop with around 50 participants was organised to identify scientific evidence that was not identified by the WG using the approach described in this chapter (see Event Report at https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/1026e.pdf, last accessed 22 August 2016).

- TITLE: ((bee OR bees) OR (apis AND mellifera) AND health) AND TITLE: (review) Timespan: 2000–2015. Search language=Auto
- TITLE: ('honeybee') or loss*) Timespan:2005–2015 Search language=Auto
- TOPIC: (bee) AND TOPIC: (ecosystem) AND TOPIC: (review) Timespan: 2000–2015. Search language=Auto

The main multidimensional characteristics of a healthy managed honeybee colony were listed and used as a basis for discussion by multidisciplinary groups of experts (HEALTHY-B and MUST-B working groups). A managed healthy honeybee colony has been described and three overarching concepts were identified (see Section 3.1).

2.1.2. Identification of indicators and factors

To identify indicators and factors for which data have been collected in field surveys in addition to the expert contribution gathered in the context of the preparation of this paper, the following data sources were consulted:

- national or international bee health monitoring programmes – EPILOBEE, BeeNet, APENET, German Bee Monitoring Project, COLOSS project (e.g. Bee Book), UK's Bee Health programme[7];
- publications identified using searches described in Section 2.1.1;
- Web of Science using the search string 'honeybee*' AND health AND monitoring, 2000–(March) 2015, English;
- publications and/or scientific reports of projects or working groups provided by experts, in particular papers published between March 2015 and June 2016.

Beekeeping practices can differ between Europe and other continents (e.g. more intensive movement of colonies in the United States). Therefore, screening of the scientific documents to identify indicators and factors focused on the European situation and did not include reports from, for instance, the US monitoring programme BEEinformed. However, studies from outside the EU are taken into account when scoring indicators or factors (see Section 2.2) if the context of the study is relevant to the European situation.

An overview of the identified indicators and factors from field surveys was made and was used as a basis to generate overviews presented in the form of mind maps on indicators and factors for colony attributes, external drivers or colony outputs. The structure of the mind maps is based on the life-history theory (Fabian and Flatt, 2012) and considers the budget of energy and material as the key drivers of all the physiological processes within a colony. Indicators and factors that could be measured under experimental conditions were also included in the mind maps, creating a toolbox that will facilitate the selection of relevant indicators and factors for a given objective (e.g. field surveys, risk assessment or modelling).

2.1.3. Identification of variables and methods

For selected indicators or factors, one or more variables were identified by consulting the published scientific literature, bee experts and experts from other relevant fields. The main methods were listed and experts identified the 'preferred' method depending on its suitability for harmonisation and use in multifactorial field surveys in most Member States.

2.2. Procedure for selection of indicators and factors

2.2.1. Procedure and scoring system used

After identifying the indicators and factors, a functional or operational definition was provided for each indicator and factor (Figure 2). The indicators and factors were then scored (high or low) for their relevance to the health status of a managed honeybee colony or relevance an understanding of the context of a managed honeybee colony, respectively (see definitions in Table 2). Only indicators and factors with a high score on 'relevance' were subsequently assessed for their technical feasibility in the context of multifactorial field surveys (high or low score) (see definitions in Table 2). Technical

[7] https://www.gov.uk/guidance/bee-health (last accessed 1 June 2016).

feasibility is assessed in relation to the type of study considering: (i) the problem formulation and objectives of the study (e.g. development of a surveillance system based on monitoring of a minimum set of variables); (ii) the resources available; and (iii) the operators involved in the data gathering (beekeepers, inspectors, laboratories, etc.). Only the indicators and factors with high scores for technical feasibility in field surveys were assessed in terms of their priority for inclusion in field surveys (high, medium or low score) (see definitions in Table 2). The outcomes are described in the main text (TOR2 in Sections 3.2, 3.3 and 3.4) and in detailed tables in Appendix B.

Indicators and factors with an H-HH score (H-HH[8] meaning High relevance, High technical feasibility and High priority; Table 3) were considered in TOR3. Variables were identified to characterise each indicator or factor and how to measure and report these was analysed. For each indicator or factor, one variable was identified for preferential use in field surveys that could be applied in several Member States with the objective to facilitate harmonisation and comparison of data in time and space within Europe. The outcomes are described in the main text (TOR3 in Sections 3.2, 3.3 and 3.4).

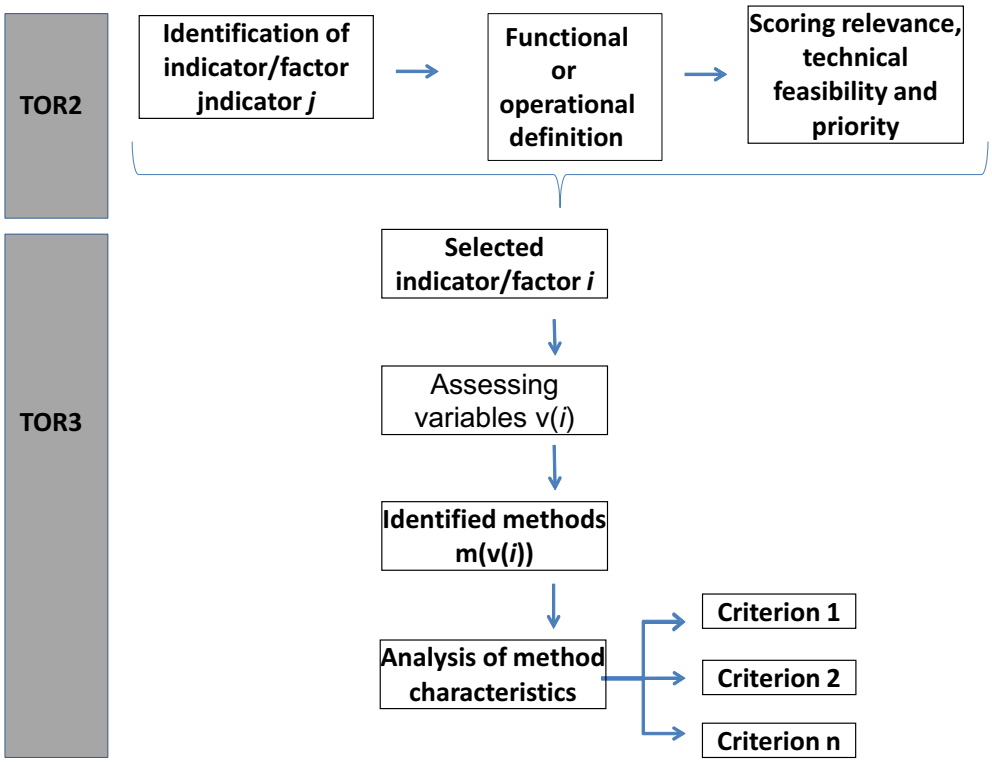


Figure 2: Approach followed to select indicators and factors (TOR2) and to identify and analyse methods that could be used to measure and report the selected indicators and factors (TOR3)

Table 2: Criteria and descriptions used in the assessment of bee health indicators/factors

Criterion	Expert judgement	Description
Relevance to assessing the health status of a managed honeybee colony (indicators) Relevance to understanding the context of a managed honeybee colony (factors)	High	There is robust scientific evidence suggesting an association of the indicator/factor with bee health
	Low	There is no or little scientific evidence suggesting an association of the indicator/factor with bee health

[8] The hyphen differentiates the score on the relevance to bee health (which is independent of the study objective) from the scores on technical feasibility and priority for inclusion in field surveys (which may vary when the study objective changes).

Criterion	Expert judgement	Description
Technical feasibility in field survey	High	Measurement of the factor/indicator is or could be routinely applied by a beekeeper within the context of a field survey
	Low	Measurement of the factor/indicator cannot or could not be routinely applied by a beekeeper within the context of a field survey
	NA	Not applicable because not assessed
Priority indicator/factor in field survey	High	Experts consider the ratio between the benefit of the data collected on the indicator/factor and the efforts in (terms of resources) to be high. In addition, the indicator/factor is highly relevant to most Member States and in most conditions[(a)]
	Medium	Experts consider the ratio between the benefit of the data collected on the indicator/factor and the efforts in (terms of resources) to be medium. In addition, the indicator/factor is highly relevant to some Member States/regions or in some specific conditions
	Low	Experts consider the ratio between the benefit of the data collected on the indicator/factor and the efforts in (terms of resources) to be low. In addition, the indicator/factor is highly relevant to few Member States/regions or in very specific conditions
	NA	Not applicable because not assessed

(a): Referring, among others, to geographical, climatic, environmental or beekeeping management conditions.

Table 3: Combinations of scores used for the selection of indicators/factors to be measured in a field survey

Score	Relevance to assessing colony attributes (indicators) or external drivers (factors)	Technical feasibility in field survey	Priority indicator/ factor in field survey
H-HH[(a)]	High	High	High
H-HM	High	High	Medium
H-HL	High	High	Low
H-L	High	Low	Not assessed
L	Low	Not assessed	Not assessed

(a): The score H-HH is highlighted in green as the indicators and factors with these score are taken forward in TOR3, whereas the other indicators and factors not.

2.2.2. Data collection in field surveys

In order to proceed towards a holistic risk assessment on bee health in the future (the objective of EFSA's MUST-B project), it is required to promote EU-wide bee health monitoring covering all colony attributes, external drivers and colony outputs. This was one of the recommendations made by EFSA (2014), which could be achieved by: (i) a large-scale multifactorial field survey collecting data from different Member States over a few years, or (ii) combining data from smaller field surveys carried out in parallel in different Member States. Since it is unlikely that a new pan-EU bee health monitoring study will be launched in the near future and several Member States already have ongoing bee health field surveys (see some examples in Table A.1, Appendix A), it is probably more efficient to facilitate harmonisation of indicators, factors, variables and methods to allow the merging of several (national) data sets and subsequent meta-analyses. Every Member State can keep the specific objectives of its field surveys but the use of tools from a common toolbox should facilitate data exchange and comparison of (at least some) results, which is often not possible at the moment.

As described in the TORs of this mandate, the AHAW Panel focuses on large field surveys when selecting tools from the toolbox to guarantee that the tools are applicable across the EU. A field survey is seen as a basic (as simple as possible) procedure to collect data aimed at understanding the health

status of a honeybee colony and its variation in time and space. It is carried out through the implementation of different types of methods including observations, measurements and interviews. In this opinion, the AHAW Panel does not provide a typology of field surveys nor performs a survey design. This is to allow the use of the toolbox in ongoing Member State field surveys that might have different objectives, available resources and/or sampling/analysis capacity. However, some of the Panel's considerations are mentioned below in relation to the design and implementation of a survey. They are directly linked to:

- the problem formulation: in general terms or from specific perspectives;
- the spatial and temporal extent and resolution of the study to develop;
- the variability of the sampling variables and the precision of the estimates required for the analyses.

It is recommended that many indicators and factors are measured at least three times during a year: after winter (1–2 weeks after bees start foraging but before the first big nectar flow), during summer (active season) and before winter (when the colony is preparing for winter). The flowering of *Salix* spp. was suggested by the workshop participants in an attempt to guide when the 'after winter' inspection should take place although no publication was found to support this idea. An alternative could be to check whether 'cumulative day degrees' could be used to define the start of the beekeeping season in a more harmonised way across the EU. It is considered better than using fixed dates because the length of the beekeeping season is different in northern versus southern Europe. However, it is clear that more frequent data collection will enrich the data set and is recommended whenever possible. The exact timing of the measurements has to be defined based on the objectives of the field survey. More guidance on the 'sampling frame' is provided in Section 5. Furthermore, two people are required to collect information from a given hive, particularly for the indicators (one checking the hive and the other one documenting the observations).

Furthermore, special attention should be given to the different actors involved in the data collection linked to field surveys. They should be informed about the objectives and reasons for performing the field survey, about the survey protocol including frequency of the honeybee colony inspections, the use of preferably standardised sampling and measurement methods, reporting methods and data protection issues. At least two different actors should be involved:

- Beekeepers are the people managing the colony throughout the year. They provide information and data required in a field survey. It is crucial that the beekeeper receives targeted training to accurately obtain and report data.
- Inspectors or operators are people assisting the beekeeper in collecting particular samples and/or data for the survey; they might also be involved in the sample collection and/or analysis depending on the questions to be addressed and the local and regional beekeeping task organisation. These people are usually appointed by the survey coordination team, are specifically trained for sampling and ensure harmonisation of data collection between different colonies, apiaries and regions.

2.3. Workshop

For TOR1–3, involvement of hearing experts and the organisation of a workshop on bee health were important to identify relevant scientific evidence that was not identified by the WG, to identify any that is not clear 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 anyone with relevant expertise who wanted to participate in the workshop. Around 55 experts registered their interest in participating, and around 30 were selected who fulfilled the eligibility criteria. Around 20 other experts were invited directly by EFSA, including the WG members. The workshop took place on 13–14 April 2016 and was built around breakout sessions that covered the different chapters of TOR1–3. Detailed discussions took place on comments provided by the participants and selected by the WG for their relevance to be discussed by a broader expert group. An event report is available on the EFSA website.[9] The collected information was considered by the WG when finalising the draft scientific opinion.

[9] https://www.efsa.europa.eu/it/supporting/pub/1055e (last accessed 11 July 2016).

3. Assessment

3.1. Identification of the colony attributes, external drivers and colony outputs (TOR1)

3.1.1. Characteristics of a managed healthy honeybee colony

A colony of managed honeybees was defined as an *Apis mellifera* bee population kept by a beekeeper with the presence of a given queen. Replacement of the queen by a natural process or by a beekeeper is considered to result in a new colony because it changes the genetics of the population.

Based on a scoping of the scientific literature (see Section 2.1.1) and subsequent discussion by WG members and hearing experts representing different stakeholders, it was concluded that the characteristics of a healthy managed honeybee colony are:

- an adequate[10] size, demographic structure and behaviour in relation to the annual life cycle of the colony and the geographical location;
- an adequate[9] production of bee products in relation to the annual life cycle of the colony and the geographical location;
- provision of pollination services.

These are characteristics of a healthy honeybee colony (but should not be seen as a definition) that lead to the identification of three overarching concepts: colony attributes, external drivers and colony outputs (see definitions in Table 1, Section 1.2). Colony attributes reflect the health status of a managed honeybee colony. External drivers that affect the health status and colony outputs express the productivity of a managed honeybee colony. Production of bee products and the provision of pollination services were included because these are the drivers for beekeepers to manage a honeybee colony. The concepts are further defined in Sections 3.1.2–3.1.4 below and the relationships between them are presented in Figure 3.

It is suggested that indicators, such as *Varroa destructor*, and molecular markers. such as vitellogenin, may be predictive markers for winter survival (Dainat et al., 2012b; Ravoet et al., 2013; Smart et al., 2015). Collecting information on such indicators is required to validate their use as a health marker across Europe and their possible future inclusion as a characteristic of a healthy managed honeybee colony.

3.1.2. Colony attributes

Five colony attributes have been distinguished that should be analysed when assessing the health status of a honeybee colony (Figure 3):

- queen presence and performance;
- behaviour and physiology;
- demography of the colony;
- in-hive products;
- disease, infection and infestation.

Each colony attribute is described in Section 3.2 and mind maps are presented to give an overview on all the indicators that were identified and scored for their relevance in relation to each colony attribute, as well as for their technical feasibility and priority for implementation in a field survey.

3.1.3. External drivers

Three different external drivers have been distinguished (Figure 3):

- environmental drivers;
- resource providing unit (RPU);
- beekeeping management practices (BMP).

Each external driver is described in Section 3.3 and mind maps are presented to give an overview on all the factors that were identified and scored for their relevance in relation to each external driver, as well as for their technical feasibility and priority for implementation in a field survey.

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

3.1.4. Colony outputs

The colony outputs are considered in terms of service provision of the managed honeybees to the ecosystem. Only two of the main ecosystem services directly provided by the managed honeybees are addressed in this opinion (Figure 3):

- the pollination services provided by the honeybee colony in terms of regulating ecosystem services (= regulating service);
- the products harvested by the beekeeper, the hive rental service and the live honeybees extracted from the honeybee colony in terms of provisioning ecosystem services (=provisioning service).

The colony outputs are described in Section 3.4 and a mind map is presented to give an overview on all the indicators that were identified and scored for their relevance in relation to the colony outputs, as well as for their technical feasibility and priority for implementation in a field survey.

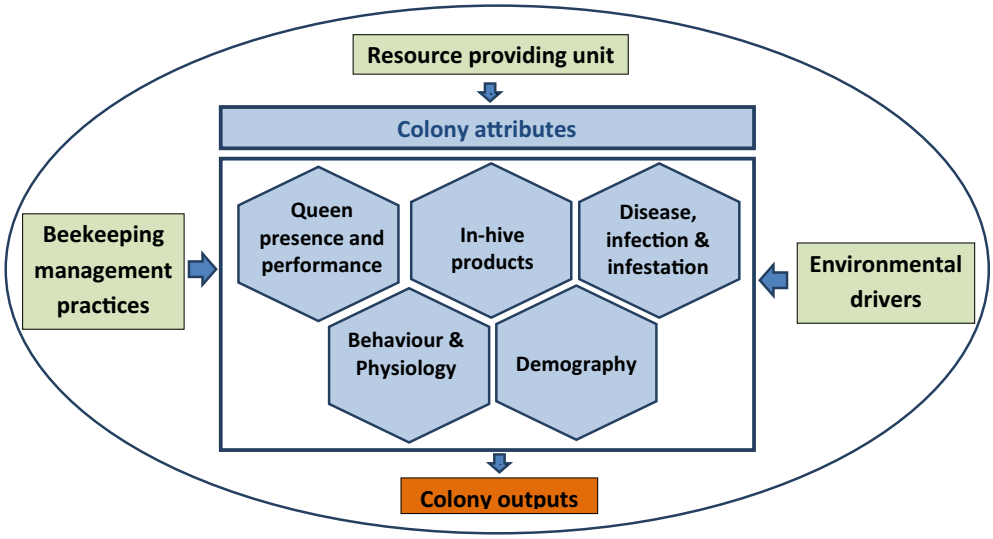


Figure 3: Colony attributes (elements in blue), external drivers (elements in green) and colony outputs (elements in orange) to be considered in a multidimensional assessment of the health of a managed honeybee colony

3.2. Colony attributes reflecting the health status of a managed honeybee colony (TOR2–3)

3.2.1. Queen presence and performance

3.2.1.1. Identification of indicators related to queen presence and performance (TOR2)

An assessment of the health status of a managed honeybee colony should include analysis of the presence and performance of the queen because this influences the size, structure and survival of the colony (see Section 3.1.1). The indicators measuring queen presence and performance were identified using the methodology described in Section 2.1.2. All indicators are presented in Figure 4 and detailed information is provided in Appendix B, Table B.1. The paragraph below briefly describes indicators, in particular those with high scores.

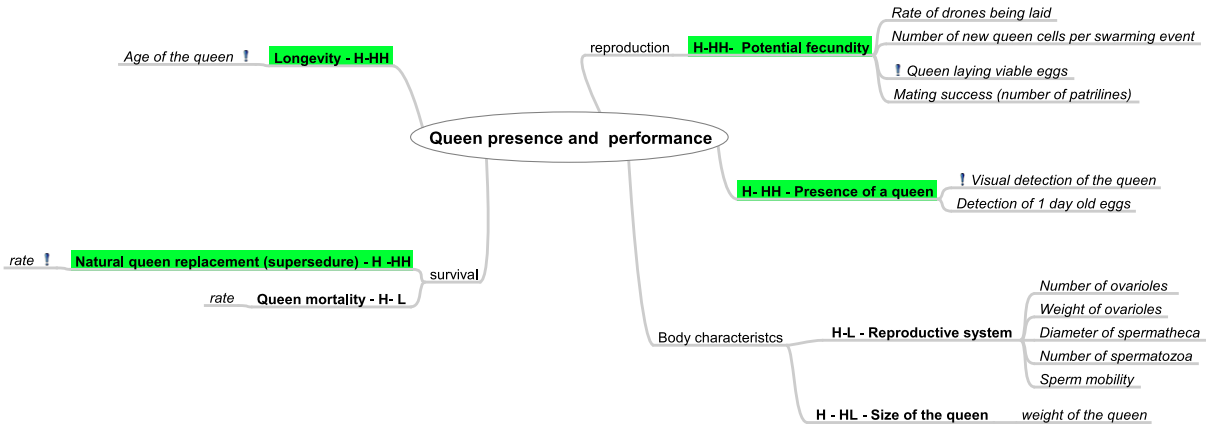
(i) Relevance of queen presence and performance indicators to the bee health status of a colony

The colony will not survive without the presence of (or the ability of the colony to produce) a queen (Winston, 1991). Excessive queen mortality or replacement of the queen by the colony (e.g. a supersedure queen) could indicate that the colony is not healthy and/or the queens being produced are of chronically low quality (Page and Peng, 2001). The longevity of the queen, as measured by her age, affects her reproductive capacity and, therefore, the likelihood that she will be replaced (Tarpy et al., 2000). For example, her potential fecundity should be sufficient to lay viable fertilised and unfertilised eggs, with appropriate worker/drone proportions and a rate that is typical for the season

(Fyg, 1964). Lastly, included in potential fecundity is a consideration of whether or not the queen mated with sufficient males on her mating flight (mating success), as this is known to affect the vigour and survival of the colony (Tarpy et al., 2013; Mattila and Seeley, 2014).

(ii) Technical feasibility and priority to include queen presence and performance indicators relevant to bee health in field surveys

For the indicators linked to bee health, the technical feasibility and priority for inclusion in a field survey were assessed. Direct analysis of queen mortality is considered to have too low a feasibility to be included routinely in field surveys as it is often very difficult to find a dead queen in a colony and because it is a common practice for beekeepers or for the colony to replace a queen before 'normal' mortality occurs. The presence of a queen and the potential fecundity are very important indicators of queen bee health and, likewise, colony health, as they determine the demography, survival and strength of the colony. They can both be assessed by a beekeeper during routine hive inspections. Additionally, information on the longevity of the queen, as determined by her age, is relevant when assessing the potential fecundity of the queen and, indirectly, queen mortality, and can easily be provided by beekeepers, particularly when the queen is marked and records are kept. Keeping records of the queens per hive within an apiary facilitates the beekeeper to assess the natural queen replacement (supersedure), which could be an early signal of impaired health if it occurs at abnormally high frequencies.



H-HH, indicators with a High link with bee health, High technical feasibility and High priority; **H-HL**, indicators with a High link with bee health, High technical feasibility and Low priority; **H-L**, indicators with a High Link with bee health and Low technical feasibility; **!**, recommended variable to assess the corresponding indicator. The score H-HH is highlighted in green as the indicators with this score are taken forward in TOR3, whereas the other indicators not.

Figure 4: Mind map queen presence and performance – identified indicators and corresponding scores

3.2.1.2. Methods and tools to measure indicators related to queen presence and performance (TOR3)

Table 4 provides an overview of different variables and methods to measure the indicators 'presence of a queen', 'potential fecundity', 'natural queen replacement (supersedure)' and 'longevity of a queen'. All the methods can be implemented by a beekeeper during every routine inspection of a hive. Labelling the queen facilitates monitoring of its age and replacement. It is recommended that data on these indicators are collected at least three times a year: after wintering, during the active beekeeping season and before wintering. The text below compares the variables and corresponding methods per H-HH indicator and suggests which variable/method is considered most suitable for implementation in field surveys. Detailed information on the suggested methods is provided in Appendix C (Tables C.1–C.3), aiming to further facilitate their harmonisation across the Member States.

Presence of a queen

It is recommended to detect visually the queen in a colony by checking through the hive combs, because seeing the queen is the ultimate proof that she is present and alive. A marked queen is easier to detect, and for this reason, this practice of marking is highly recommended, as mentioned

previously. In case the queen cannot be seen, visual detection of young (e.g. 1–3-day-old) eggs can be used as an indication that she is present. Ideally, a beekeeper would both detect the queen visually and see young eggs.

Potential fecundity of a queen

It is recommended to assess the potential fecundity of the queen by observing in a qualitative fashion an amount of all the stages of worker brood (eggs, larvae and pupae) in the hive that is expected for the time of year because it indicates that the queen is fertile and the colony is successfully rearing viable eggs (e.g. has sufficient resources) through all the stages of development. Visually identifying the queen laying the eggs is a less preferred method because a beekeeper is less likely to spot this when the hive has been opened. Determining the mating success of the queen, which would require microsatellite analysis, is considered possible only in a research setting. However, it should be noted that a sufficiently mated, healthy queen should be laying viable eggs, and that the brood pattern should be solid (see 'brood consistency' in Section 3.2.2).

Longevity of a queen

Labelling the queen in conjunction with record keeping is the only method available to determine her age. Marking the queen takes only a few minutes once a new, unlabelled queen is found, and can be done by a beekeeper using either number tags or paint pens. Using international code colours can facilitate the process of determining queen age (Human et al., 2013); otherwise the beekeeper can rely on his/her own record keeping. Every time the hive is inspected, it should be assessed whether the queen identified and marked during the last visit is still present or whether she has been replaced by a new (unmarked) queen. If the beekeeper has replaced the queen, this should be noted. If regular queen replacement by the beekeeper takes place regardless of status/performance/age of current queen, this variable is no longer meaningful.

Natural queen replacement (supersedure)

Similar to the assessment of queen longevity, labelling the queen in conjunction with record keeping is the only method available to determine the rate of natural queen replacement, which is when the colony 'decides' that a queen needs to be replaced. Natural queen replacement (supersedure) should be reported as the number of new queens detected in 2 years and does not include those queens that were introduced into the colony by the beekeeper. New queens are recommended to be labelled as explained in the previous paragraph. As stated above, if the beekeeper has replaced the queen, this should be noted. If regular queen replacement by the beekeeper takes place, then the hive never has the opportunity to express queen supersedure, making this variable no longer meaningful.

Table 4: Measurement of selected indicators on queen presence and performance

Indicator	Variable [unit][(a)]	Method[(a)]
Presence of a queen	Visual detection of the queen [Y/N]	Visual verification by checking through combs and in the walls of the hive
	Detection of fresh eggs [Y/N]	Visual verification of presence of 1-day-old eggs
Potential fecundity	Queen laying viable worker eggs [Y/N]	Qualitative visual identification of worker pupae, larvae and eggs
	Queen laying viable worker eggs [Y/N]	Visual identification of the queen laying
Longevity of a queen	Age of the queen [months]	Queen labelling and record keeping
Natural queen replacement (supersedure)	Colony rate of queen replacement [number of supersedure queens/2 years]	Queen labelling and record keeping

(a): Variables, methods, implementation and timing most suitable for implementation in field surveys across the EU are highlighted in green. They can all be performed by beekeepers, preferably at least three times a year: after winter (e.g. 1–2 weeks after bees start foraging, but before first big nectar flow), during summer (active season) and before winter (when the colony is preparing for winter) (see Section 2.2.2). However, it is clear that more frequent data collection will enrich the data set and is recommended whenever possible (see 'sampling frame' in Section 5). The exact timing of the measurements has to be defined based on the objectives of the field survey. Details on these methods are given Tables C.1–C.3.

3.2.2. Demography of the colony

3.2.2.1. Identification of indicators related to demography of the colony (TOR2)

A honeybee goes through different life stages during its development: egg, larvae, pupae and adult bee. There are three main castes of adult bees: the queen, drones and workers. The role of the queen is described above (see Section 3.2.1). The drones mate with the queen of another colony and are consequently important for the local bee population, but they are of less direct benefit to their own colony. They do not participate in the production of the colony, but they do participate in the consumption of honey. The adult workers perform virtually all the tasks of the colony, except the laying of eggs, which is performed by the queen (Winston, 1991). The workers' activities have a temporal basis, with in-nest tasks being performed by younger workers (until around 3 weeks old) and outside tasks being done by older workers. Among the in-hive bees, the nurses are especially crucial, because they must take care of the immature stages (see Section 3.2.4). Some indicators of a healthy honeybee colony are relevant to the demography of one particular life stage, whereas other indicators are relevant at the colony level in general (see Figure 5). An assessment of the health status of a managed honeybee colony should include analysis of the demography because this influences the size, structure and survival of the colony (see Section 3.1.1). The indicators measuring demography were identified using the methodology described in Section 2.1.2. The indicators are presented in Figure 5 and detailed information is provided in Appendix B, Table B.2. This section focuses on indicators that can be measured when a colony is inspected by a beekeeper and/or inspector, whereas the survival of a colony is dependent on several indicators and should be measured over time. Therefore, 'survival' is considered an outcome of the analysis of multiple indicators. The paragraph below briefly describes 'demography' indicators, in particular those with high scores.

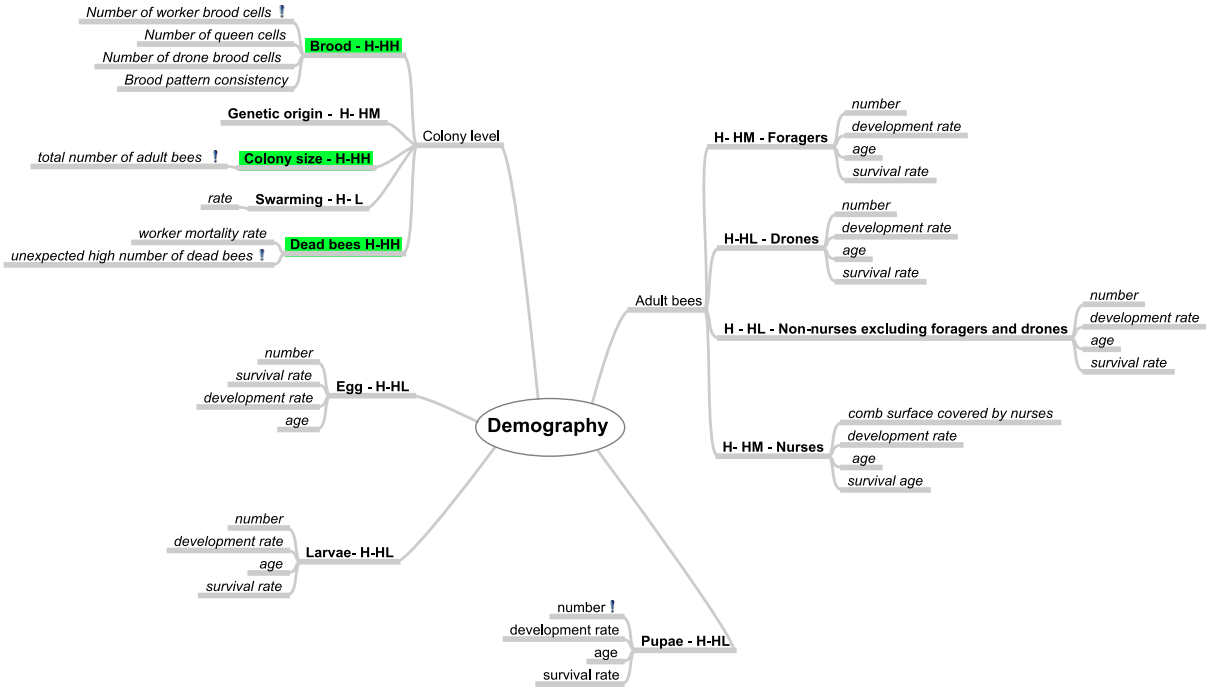
Relevance of demography indicators to bee health

Brood demography refers to the amount, survival and development of worker brood and queen brood in a hive. In particular, the amount of brood is a key indicator for the development and survival of the colony because it represents the future bee population of the colony (Winston, 1991). The amount of brood follows an annual cycle and brood must be present during the whole colony development cycle except during queen succession, certain periods in winter and periods in summer with very high environmental temperatures, which might occur in southern Europe. The brood pattern consistency (or brood solidness) is a qualitative variable interesting to include in a survey. A 'spotty' brood (e.g. > 10% of empty cells) is a sign of a problem of brood quality which could be due to low sperm quality (Collins, 2000; Delaplane et al., 2013b), disease, etc.

A reduced colony size (number of workers) in suitable environmental circumstances indicates a possible health problem in the colony. The presence of dead bees in the vicinity of the hive, in the hive entrance and in the bottom of the hive may be indicative of a health problem affecting the colony.

However, a reduced colony size could also be due to a recent swarming event. Swarming happens when the colony is densely populated and lacks space to expand within the hive. Swarming during the active period of the colony life cycle in a given area according to the availability of environmental food resources is a symptom of good health. There is variation between colonies and bee races to produce swarms more than once a year. The multiplication of colonies by swarming is a benefit for the species in general and the local bee population, in particular, because the number of colonies increases. However, a higher number of swarms per year than is normal for a given bee subspecies or swarming at a wrong time of the life cycle can also be a sign of poor health. Reduction of the colony size due to swarming should not be confused with reduced survival of the colony, which reflects a reduction in the number of workers due to mortality. There is evidence that one part of the variability in the survival of honeybee colonies is connected to local adaptivity, representing differences in climate, vegetation, infestation pressure and colony management (Spivak and Downey, 1998; Danka et al., 2013; Büchler et al., 2014; Büchler et al., 2010). It is generally considered that the best locally adapted bee colonies are subspecies (geographical races) of native bees that have developed over millennia (Meixner et al., 2010; Büchler et al., 2014). The structure of the colony, determined by the demography of the ratio between nurses and foragers, is also related to its health status because honeybee colonies function as super organisms (see Section 3.2.4). Each worker bee participates by performing specific tasks necessary for the good functioning of the colony, which change during its life cycle (see previous paragraph). The timing of the transition from one life stage to another is highly flexible and is determined by the needs of the colony (Robinson et al., 1992; Huang and Robinson, 1996), although

rapid maturation accelerates the failure of chronically stressed honeybee colonies (Perry et al., 2015). Taken together, all indicators describing the demography of the colony are linked to bee health.



H-HH, indicators with a High link with bee health, High technical feasibility and High priority; **H-HM**, indicators with a High link with bee health, High technical feasibility and Medium priority; **H-HL**, indicators with a High link with bee health, High technical feasibility and Low priority; **H-L**, indicators with a High Link with bee health and Low technical feasibility; **!**, recommended variable to assess the corresponding indicator. The score H-HH is highlighted in green as the indicators with this score are taken forward in TOR3, whereas the other indicators not.

Figure 5: Mind map demography of the colony – identified indicators and corresponding scores

Technical feasibility and priority to include demography indicators relevant to bee health in field surveys

The technical feasibility and priority of the inclusion in a field survey was assessed for each indicator (Appendix B, Table B.2). Brood demography and colony size are considered key indicators to describe the demography of the colony and its potential to produce honey and provide pollination services. Quantifying the brood cells and the living adult worker bees in a colony can be undertaken by a beekeeper during a standard hive inspection. Several methods, with varying levels of precision, are already applied to estimate the number of workers in a colony, such as weighing or evaluating the surface of combs covered by bees (Imdorf et al., 1987; Delaplane et al., 2013b; Costa et al., 2012; Odoux et al., 2014; Porrini et al., 2016). However, all methods might underestimate the real colony size because by opening the hive for evaluations, a certain number of workers will not be counted, and the method also fails to take into account the number of foraging bees that had left the colony at the time of measurement (see below).

Collecting information on dead bees by assessing the worker mortality rate[11] would provide relevant information on the health status of the colony. It would give more accurate information supplementing the analysis of the colony size over time. However, this variable is difficult to assess in a quantitative way in a field survey by beekeepers. By contrast, the presence/absence of an unexpectedly high number of dead bees could be assessed in field surveys by visually inspecting the presence of an abnormal number of dead bees in or just in front of the hive. In cases where unexpectedly high numbers of bee corpses are found, quantification is required. Also determination of

[11] Discussion with a large group of experts would be required to determine a clear and broadly accepted definition since several definitions can be found in the scientific literature.

the swarming rate[12] would improve understanding of the colony size evolution over time. However, this is not feasible to implement because it would require very frequent visits to the hive during the swarming season to identify signs of swarming events, which is not always possible for the beekeeper. To prevent swarming some beekeepers remove the queen cells from the combs (see Section 3.3.2 for more information), so this practice could also be recorded if a better overview of the colony dynamics is required.

Given the large movement of queens in Europe (of both intra- and extra-EU origin) by beekeepers who keep specific bee subspecies, native bee populations are more or less introgressed by other subspecies. Moreover, climate change can directly affect flowering dates (e.g. Bock et al., 2014) and increased intercontinental trade might lead to the entry of exotic bee pests and diseases, which could have a negative effect on subspecies whose colony development cycle is not fully adapted. Determination of the genetic origin of a bee subspecies using molecular markers, such as microsatellites (Francis et al., 2014), could be interesting to include in a field survey in order to understand the demography of the colony in a given context. However, it is expensive and hence more feasible under experimental settings.

Every bee caste (queen, workers or drones) is important for the colony and a complete and detailed picture of colony dynamics would only be possible by assessing each developmental stage of the castes (eggs, larvae, pupae and adults). However, this assessment is feasible only under research settings, and therefore, only the demography of nurses and foragers is considered, since they feed the larvae and collect food for the colony, respectively. The ratio of nurses to foragers can give an indication of the health of the colony, however, more detailed studies are needed to calculate this ratio, because determining the exact number of each category is feasible only in an experimental setting. Beekeepers could perform a qualitative assessment of the foraging activity (see Section 3.2.4) and check if appropriate cover of the brood by nurses is available according to local circumstances. Nurses are not morphologically different from other workers, and it is generally considered that the workers covering the brood (eggs, larvae, pupae) are mainly nurses (Winston, 1991), which can lead to an overestimation of numbers because some non-nurses will be counted as nurses. When the in-hive temperature is too low, it is expected that 100% of the brood comb will be covered by nurses. If the temperature is above around 30°C it is likely that only low numbers of nurses are present on the brood comb. If the brood coverage by nurses is not appropriate in terms of the outside temperature, this is considered as an atypical behaviour of worker bees (see Section 3.2.4) or an insufficient number of nurses, which might be the sign of a health problem. Even though drones are of less direct benefit for their own colony, the low production of drones can be a sign of unhealthy colony, and the same applies for to the production of much drone brood late in the season. Analysis of the demography of eggs, larvae, pupae, drones and non-nurses could be included in detailed studies to enrich the data set or to answer specific research questions.

3.2.2.2. Methods and tools to measure indicators related to demography of the colony (TOR3)

Table 5 provides an overview of different variables and methods to measure the indicators 'colony size', 'dead bees' and 'brood demography' (more details are available in Appendix C, Tables C.4–C.12). All the methods can be implemented by a beekeeper during every routine inspection of a hive. Preferably, the data should be collected by two people, one measuring the indicators and the other recording the data. It is recommended to collect data on these indicators at least three times a year: after wintering, at the peak of activity of the colony during beekeeping season and before wintering. Considering the variability due to the different climatic zones in the EU, the precise choice of the dates should be made at the national/local levels. The text below compares the variables and corresponding methods per indicator and suggests which variable/method is considered most suitable for implementation in field surveys. The variable outcome should always be reported together with the method used. Detailed information on the suggested methods is provided in Appendix C (Tables C.4–C.11), aiming to further facilitate their harmonisation across the Member States.

Colony size

It is recommended to determine the colony size by visually estimating the total amount of comb surface[12] covered by adult bees. Input on the type of comb would also be needed. The collected data

[12] In case there are only a few bees on the side of a comb, the surface of that comb that is covered by bees should be estimated (e.g. 1/4 of comb) and the total number of combs covered by bees in the hive reported (e.g. 4.5 combs).

together with the average number of bees per cm^2 (e.g. table 2 in Delaplane et al., 2013b) will then allow calculating the total number of adult bees in the hive during the data analysis step. Variation between measurements can be large due to the subjective nature of this technique (i.e. relies on visual observation). Variability in data collection methods may be reduced if some training is provided to the beekeepers on how to accurately apply the technique and if it is applied simultaneously by two people, who independently score the comb coverage and further calculate the average of their observations. Assessment of the colony size should be done when most, if not all, bees in the population are present in the hive (Delaplane et al., 2013b) (either by performing the assessment in the early morning/late afternoon, or by closing the hive exit the day before to keep all the bees inside). Colony size can also be determined by weighing the hive with and without adult bees, by brushing the combs and transferring the bees into a temporary hive (Delaplane et al., 2013b). This method requires the use of a scale and it is more likely to disturb the colony, but has less variability between measurements.

Brood demography

The number of brood cells (eggs, larvae and pupae) can be estimated by calculating the total amount of comb surface covered by brood cells. Input on the type of comb and brood density would also be needed to further derive the total number of brood cells in the hive during the data analysis step. The total surface covered by brood can also be calculated by using tools that allow a more precise estimation of the brood comb surface. For instance, the brood surface can be approximated to an ellipse, the length and width of the brood area measured, and the brood area calculated. Another possible method is to place a transparent grid in the brood comb to facilitate measurement of the brood area. Both methods require further conversion of the total comb surface to total number of brood cells, so input on the type of comb and brood density is also needed. Digital photography of the brood combs followed by image analysis using appropriate software allows measurement of the surface and also the number of brood cells. These methods provide results with less variability than the method based on visual observation, but might be difficult to implement in extensive field surveys because they require time, equipment and/or expertise that may not always be available. Tools that record the vibrational activity of the colony to monitor brood cycle are currently being developed and may represent an interesting tool for the future (see, for instance, Bencsik et al., 2015). Brood pattern consistency is an interesting qualitative variable to include in a survey.

Dead bees

The presence of an unexpectedly high number of dead bees in and/or around the hive can be assessed by a beekeeper when performing a routine inspection of the colony. A case where are many more dead bees than generally observed by the beekeeper when inspecting a colony should be reported together with an estimate on the number of dead bees observed. Determination of the worker mortality rate can be done using radio frequency identification (RFID) (Streit et al., 2003), but this is restricted to research settings.

Brood pattern consistency

Brood pattern consistency can be estimated by counting the percentage of empty worker brood cells in a defined area. A rhombus (a parallelogram of 10 $\times$ 10 cm, for example) should be placed in a central area of a frame containing brood and the number of filled (cells containing egg, larvae or pupae) and empty brood cells counted. The percentage of empty cells can then be estimated. More than 10% of empty worker brood cells could be indicative of a health problem due, for example, to low sperm viability. Digital photography of the brood combs followed by image analysis using appropriate software could also allow estimation of brood pattern consistency.

Table 5: Measurement of selected indicators on 'demography'

Indicator	Variable [unit][(a)]	Method[(a)]
Colony size	Total number of combs covered by adult bees estimated by visual observation [number of combs], [type of comb]	Visual estimation of the comb surfaces covered by bees
	Total number of adult bees derived from population weight [kg]	Weighing the hive with and without bees
Dead bees	Unexpected high number of dead bees in and around the hive [yes; no]	Visual estimation (if < 1,000 dead bees) or weighing (if > 1,000 dead bees)
	Total number of combs covered by brood cells estimated by visual observation [number of combs], [type of comb]	Visual estimation of the number of combs covered by brood cells
Brood demography	Total number of brood cells estimated by measuring the brood area [cm^2]	Estimation of the comb surface covered by brood cells by approximating comb surface to ellipses and measuring surface area
	Total number of combs covered by brood estimated by visual observation [number of combs], [type of comb]	Estimation of the comb surface covered by brood cells using a transparent grid
	Total number of brood cells estimated by digital photography [number]	Estimation of the number of brood cells using digital photography followed by image analysis
	Brood pattern consistency [%]	Estimating percentage of empty brood cells in a 10 × 10 area

(a): The variables and methods suggested for implementation in field surveys are highlighted in green. They can all be performed by beekeepers, preferably at least three times a year: after winter (e.g. 1–2 weeks after bees start foraging, but before first big nectar flow), during summer (active season) and before winter (when the colony is preparing for winter) (see Section 2.2.2). However, it is clear that more frequent data collection will enrich the data set and is recommended whenever possible. The exact timing of the measurements has to be defined based on the objectives of the field survey (see 'sampling frame' in Section 5).

3.2.3. In-hive products

3.2.3.1. Identification of indicators related to in-hive products (TOR2)

An assessment of the health status of a managed honeybee colony should include analysis of the in-hive products because they influence the energy available to the colony for its development and functioning, including the provision of bee products and pollination services. Indicators measuring in-hive products were identified using the methodology described in Section 2.1.2. The indicators are presented in Figure 6 and detailed descriptions are provided in Appendix B, Table B.3. They are subdivided into food stock (bee bread, honey and jelly) and non-food stock (propolis and wax). The paragraph below briefly describes the 'in-hive product' indicators, particularly those with high scores.

Relevance of food stock indicators to the bee health status of a colony

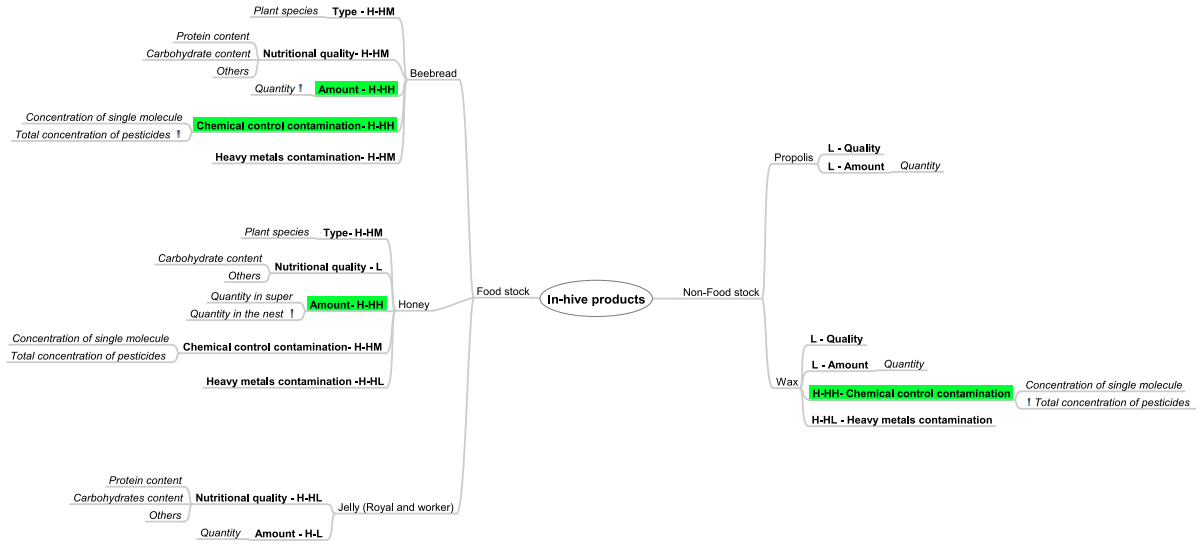
Pollen that have been completely processed for storage to prevent germination in the hive, are often referred to as 'bee bread'[13] (Winston, 1991). Bee bread is the main source of protein and lipid for honeybees and is necessary for honeybee development (Campos et al., 2008; Brodschneider and Crailsheim, 2010). The type of bee bread is determined by the diversity of the pollen in it and is related to its nutritional quality (Schmidt, 1984; Schmidt et al., 1987; Di Pasquale et al., 2013; de Groot, 1953). Pollen can differ between floral species in terms of nutritional contents (Roulston and Cane, 2000; Odoux et al., 2012), suggesting that some are pollens are of better quality for bees than others. Pollen may also provide information on the foraging source. The amount of bee bread should be related to the brood development needs of a honeybee colony during its annual life cycle; for instance, to increase the population of workers after the winter, to replace dead workers during the active/late season or to raise workers just before the end of the active season or before wintering (Winston 1991). Nectar and honeydew collected from the environment are natural carbohydrate sources for honeybees, but they are immediately deposited into the cells of the combs and are transformed into honey in 1–5 days. However, some quantities of nectar and honeydew could be consumed directly by the bees during the season, before being transformed into honey. Bees store

[13] The terms 'pollen' or 'stored pollen' are often used in publications instead of 'bee bread'.

honey for use both during the active season (to carry out all their tasks) and during wintering (to maintain the temperature of the colony). The nutritional content of honey is mainly determined by glucose and fructose (White, 1975) and is not reported to be related to bee health. It is also essential that bees ingest lipids, vitamins and minerals (Haydak, 1970), but the actual requirements and optimal levels needed by honeybees remain relatively unexplored. During a field survey, it is essential to evaluate the total quantity of honey, nectar and honeydew present in the hive.

Royal jelly is the exclusive food of the queen larvae and adult queen. It is also the basis of the food of the young worker larvae, the worker jelly (Winston, 1991). The nutritional quality of royal jelly is related to bee health because major royal jelly proteins are thought to be responsible for the specific physiological development of the queen (Salazar-Olivo and Paz-González, 2005). There should be sufficient jelly to feed the larvae but the quantity eaten by a larva is not precisely known.

In-hive products can be contaminated with molecules and/or their derivates originating from pesticides, veterinary medicines and antibiotics, and in this scientific opinion are referred to as 'chemical control contaminants'. Numerous studies have detected a range of pesticides in bee bread or in pollen sampled in a pollen trap at the entrance of the hive or in honey (see Appendix E). Pesticide contamination of food stock and wax can have a negative effect (including sublethal) on the health status of a colony (Pettis et al., 2004; Wu et al., 2011; Blacquière et al., 2012; EFSA, 2013; Simon-Delso et al., 2014). Currently, the use of neonicotinoid and fipronil is restricted[14] in the EU because health risks to bees have been identified (EFSA, 2013). Data from ongoing monitoring schemes on pesticide effects taking place in some Member States might also be used to provide more insights into the acute effects of pesticides on honeybees (see, for instance, the Wildlife Incident Investigation Scheme, UK[15]). More data are required to better understand the effects of other chemical control contaminants on bee health and the effects of multiple exposure to chemical compounds. Veterinary products, used to control infectious agents affecting the colony, may also have a negative effect on bee health (Johnson et al., 2010), especially if their use is not in line with the product specifications. Veterinary products used by farmers for livestock have also been implicated in poisoning of bees, for instance in the south of France in the past.[16]



H-HH, indicators with a High link with bee health, High technical feasibility and High priority; **H-HM**, indicators with a High link with bee health, High technical feasibility and Medium priority; **H-HL**, indicators with a High link with bee health, High technical feasibility and Low priority; **H-L**, indicators with a High Link with bee health and Low technical feasibility; **L**, indicators with a Low link with bee health; **!**, recommended variable to assess the corresponding indicator. Chemical control contaminants include pesticides, veterinary medicines and antibiotics. The score H-HH is highlighted in green as the indicators with this score are taken forward in TOR3, whereas the other indicators not.

Figure 6: Mind map in-hive products – identified indicators and corresponding scores

[14] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:139:0012:0026:EN:PDF and http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:219:0022:0025:EN:PDF (last accessed 11 January 2016).
[15] http://www.nationalbeeunit.com/index.cfm?sectionid=33 (last accessed 19 May 2016).
[16] http://www.itsap.asso.fr/downloads/publications/lettre_itsap_n11_vdef.pdf (last accessed 3 June 2016).

There is currently no direct evidence of antibiotics affecting bee health and their use is not allowed in the EU[17]; however, assessment of its presence in bee products could be included in analysis for human exposure purposes. The effect of antibiotics in the gut microbiome of a colony is not yet well understood. Initial research findings indicate that recurrent use of antibiotics might lead to the introduction of resistance genes in the gut bacteria of honeybees (Tian et al., 2012).

Besides chemical control contaminants, honeybees can be exposed to other contaminants, such as heavy metals (e.g. arsenic, cadmium, selenium or lead), especially in areas of industrial activity (Bogdanov, 2006; RM Johnson, 2015). Cases of heavy metal toxicity to bees have been described under experimental conditions (Bromenshenk et al., 1991; Hladun et al., 2013; review in RM Johnson, 2015), but relevant scientific references are not available concerning their toxicity to bees in natural conditions except in areas of industrial activity (Formicki et al., 2013).

Relevance of non-food stock indicators to bee health

Bees metabolise honey and pollen into wax, which is used to build combs (Winston, 1991). However, there is a lack of scientific data concerning the quality (composition) and quantity of wax necessary to sustain the health of the colony. Contamination of wax with pesticides (see Appendix E) has been reported, which could affect the health status of the colony (Chauzat et al., 2010; Mullin et al., 2010; RM Johnson et al., 2010; Wu et al., 2011; EFSA, 2012a; Formicki et al., 2013).

Propolis (or 'bee glue') is used by bees for different purposes, such as to block holes and cracks in the hive, cement and strengthen the comb bases, coat the nest cavity with a thin insulating layer and 'embalm' the carcasses of intruders. Propolis may also have some antifungal and antibacterial properties, which protect the nest from infection and mould (Winston, 1991; Simone-Finstrom and Spivak, 2010). However, there is no clear knowledge on propolis quality and quantity requirements in relation to the health status of the colony.

Contamination in propolis and jelly is not considered in a survey given their relatively low quantities compared with other bee products (Fleche et al., 1997). However, the quantification of residues in propolis and royal jelly could be performed under research settings.

Technical feasibility and priority to include 'food stock' and 'non-food stock'-indicators relevant to bee health in field surveys

Measuring the amount of honey, nectar and honeydew in the nest (brood chamber) and the super is mainly relevant to estimating the available carbohydrates that can be consumed by bees, in particular, to survive long periods without food flow from the environment (Seeley and Visscher, 1985). Similarly, it is also considered crucial to assess the amount of bee bread because it represents the protein source of the colony. Quantification of honey and bee bread in the hive can be done by beekeepers during a standard hive inspection. It is recognised that an evaluation of the quantity of bee bread is much more difficult than for honey (smaller amounts, spread over several combs). The accurate quantification of jelly has low technical feasibility because this substance is not stored in the hive, it is secreted by the nurses directly into the larvae cells.

It is also proposed to collect data on bee bread chemical control contamination and wax chemical control contamination in field surveys since the frequency of pesticide occurrence and their concentrations are reportedly higher in pollen and wax compared with honey (Chauzat et al., 2011; Lambert et al., 2013). However, it is recommended that honey chemical control contamination is also analysed under field conditions when possible, especially before wintering. Beekeepers can take samples and send these to a qualified laboratory. Directive 96/23/EC sets out the requirements for national monitoring for certain residues and substances in live animals and animal products including honey. Regulation 396/2005 came into force in September 2008 and extends the requirement for chemical residue monitoring in honey to include certain plant protection products. All Member States report the results of the national residue monitoring plans. It is clear that these existing data should be used as much as possible. As for other indicators, pesticide concentrations have to be analysed together with data from other indicators/factors to understand its effect on the health of a colony.

Because pollen contaminated with heavy metals has been described (Lambert et al., 2012; Formicki et al., 2013), it is recommended to measure heavy metal contamination of bee bread, particularly in industrialised areas. The frequency and/or concentrations of heavy metals in honey and wax seem to be lower, although few data are available.

[17] Directive 96/23/EC and its updates.

Assessing the botanical origin of pollen, bee bread and honey will provide information on the geographical and botanical origins of these matrices and hence, indirectly on the pollination services provided by the colony. Similarly, inclusion of the nutritional quality of bee bread is considered to be of medium priority for inclusion in field surveys because the protein content is related to the plant origin of the pollen. Beekeepers can be trained to take the required samples but analysis should be done in a laboratory. Information on the nutritional quality of jelly is seen as less informative.

3.2.3.2. Methods and tools to measure indicators related to the in-hive products (TOR3)

Table 6 provides an overview of the different variables and methods available to measure the indicators 'quantity of bee bread', 'weight of honey in the nest', 'weight of honey in the super', 'chemical control contamination in bee bread' and 'chemical control contamination in wax'. All the methods (at least the sampling) can be implemented by a beekeeper during every routine inspection of a hive. It is recommended to collect data on these indicators at least three times a year: after wintering, during beekeeping season and before wintering, except for the indicators 'chemical control contamination of wax' and 'chemical contamination of bee bread', which are suggested to be collected for analysis a maximum of twice a year at the peak of the active season and before wintering due to the cost associated with the analysis. If acute mortality of bees is detected, sampling of fresh honey (nectar) is recommended, as well as dead workers. The text below compares the variables and corresponding methods per indicator and suggests which variable/method is considered most suitable for implementation in field surveys. The outcome should always be reported together with the method and the limit of detection (LOD) and limit of quantification (LOQ) used. Detailed information on the suggested methods is provided in Appendix C (Tables C.13–C.20), aiming to further facilitate their harmonisation across the Member States. Measuring chemical control contamination outside the hive is described in Section 3.3.1.

Quantity of bee bread

The quantity of bee bread in the hive can be derived by imaginatively sorting the bee bread cells in one contiguous mass in the comb and visually estimating the proportion of comb containing this resource. Input on the type of comb and average number of cells per cm^2 is also needed to further derive the total weight of bee bread in the hive during the data analysis step. Data from the scientific literature can be used to transform the bee bread surface into weight (see Delaplane et al., 2013b). Variations between measurements can be large, mainly because small variations in the quantities in each comb might be difficult to detect, but also because some bee bread may be under honey-capped cells and therefore not identified as such, and the quantity of bee bread per cell can vary. Variability in the measurement procedure may be reduced if some training is provided and the technique is applied simultaneously by two people, who independently score the comb coverage by the resource and further calculate the average of their observations. Assessing the amount of bee bread before winter is crucial because bee bread stores are very important for the success of the resumption of development and activity of the colony after the wintering period.

The weight of bee bread can also be calculated after determining the surface by using tools, such as a transparent grid or digital photography followed by image analysis, which allow a more precise estimation of the comb surface covered by bee bread cells. Data from the literature can be used to further derive the bee bread weight (see Delaplane et al., 2013b). Although providing more accurate results (i.e. less variability between measurements), it might be difficult to implement these methods in extensive field surveys because they can be time consuming and the equipment/expertise needed may not always be available.

Quantity of honey, nectar and honeydew in the nest

The quantity of honey in the nest can be assessed by estimating the proportion of comb coverage by this resource. Input on the type of comb and cell density would also be needed to derive the total weight of honey in the hive during the data analysis step. If the honey is harvested, it is considered as an output of the colony (see Section 3.4 for more details). This technique can be easily applied in field surveys because it does not require the use of specific tools, does not involve training and it is not time consuming. Assessment of the quantity of honey in the nest should be done at least three times a year: after wintering, during the active beekeeping season and before wintering. Assessing honey stores before wintering is highly recommended because they have a major influence on the success of the overwintering period.

The weight of honey can also be determined by using equipment, such as a transparent grid or digital photography followed by image analysis, which allow a more accurate estimation of the comb surface covered by honey cells. Although providing more accurate results (i.e. less variability between measurements) it might be difficult to implement these methods in extensive field surveys because they can be time consuming, and the equipment/expertise needed may not always be available.

The evolution of the weight of the hive over time could also be realised using an automatic permanent scale under the hive, but this is more related to field experiments. Changes in weight can be loosely correlated with variations in the quantity of honey (some kg or tenths of kg) which are much higher than variations in colony size (1 kg = 10,000 workers), except in some special circumstances (e.g. sudden depopulation due to swarming).

Chemical control contamination in bee bread

Determining the contaminant concentration in bee bread (processed pollen) comprises two steps: (i) sampling of bee bread or pollen by a beekeeper or a bee inspector, and (ii) chemical analysis by an analytical laboratory. There is lack of data on the stability of pesticide molecules in pollen and in bee bread at the same location over time. There also might be differences in the degradation rate of pesticide compounds, depending on the family. Pesticide analysis of bee bread collected from the hive combs provides information on the exposure of bees inside the hive to pesticides, whereas analysis of pollen collected via traps provides only information on the environmental pesticide contamination and the exposure of the foragers during the 3–5 days previously. Therefore, sampling of bee bread (inside the hive) is recommended for inclusion in field surveys. Further optimisation of laboratory methods might result in lower quantities of bee bread/pollen being required, facilitating sampling on a more frequent basis (Wiest et al., 2011; Yáñez et al., 2014). Some key aspects of sampling practices are provided in Table C.19. The analysis step is carried out by specialised laboratories and comprises extraction and subsequent identification of contaminants in the sample using validated methods. Multiresidue techniques, such as the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe), can be used for the extraction and identification of a wide range of contaminants in one analytical process (Bargańska et al., 2014). This method can be used to identify environmental contaminants, pesticides and veterinary drugs belonging to different chemical classes not only in bee bread, but also in other matrices, such as pollen, honey, honeybees and wax. There may be a diversity of pesticide residues in different matrices, e.g. depending on their hydrophilicity or lipophilicity. The pesticides to be detected might include some non-authorised products that may be used illegally. The veterinary products should include those used by beekeepers and by farmers for livestock. The LOD and LOQ of the method vary according to the analytical technique used[18] and the chemical compound detected. It is important to analyse the largest possible number of compounds known to be used within the geographical area where the colony is located and to use the method with the lowest LOQ and LOD. The toxicity (including sublethal) of the compounds for the bees should also be taken into account (see Appendix E), as should their metabolites, which might be toxic for the bees. For the most toxic molecules, multiresidue analyses may not be sufficient if the LOD and LOQ for these molecules are too high. In that case, a single-residue analysis must be performed.

Chemical control contamination in wax

A similar approach to that used in bee bread (see above) can also be used to analyse contaminants in wax, although working with wax is not easy. The differences in sampling are given in Appendix C, Table C.20 and examples of recent LOD and LOQ values for pesticide detection in wax are provided in Appendix E.

[18] Standardisation and validation in and between laboratories is required to allow comparison of the results between labs.

Table 6: Measurement of selected indicators on 'in-hive products'

Indicator	Variable [unit]	Method	Implementation	Timing[(a)]
Quantity of bee bread	Number of combs covered by bee bread [*n* combs], [type of comb]	Visual estimation of the equivalent number or portion of combs containing bee bread (Liebefeld estimation method)	Beekeeper	After winter, during summer, before winter
	Total surface of combs covered by bee bread [cm^2]	Estimation of the comb surface containing bee bread cells using a transparent grid	Beekeeper	After winter, during summer, before winter
	Total surface of combs covered by brood cells [cm^2]	Estimation of the comb surface containing bee bread cells using digital photography followed by image analysis	Beekeeper	After winter, during summer, before winter
Quantity of honey, nectar and honeydew in the nest[(b)]	Number of combs fully containing honey, nectar and honeydew in the bee part of the hive [*n* combs], [type of comb]	Visual estimation of the equivalent number of combs containing honey, nectar and honeydew in the bee part of the hive (Liebefeld estimation method)	Beekeeper	After winter, during summer, before winter
	Total surface of combs covered by honey, nectar and honeydew in the bee part of the hive [cm^2]	Estimation of the comb surface containing honey, nectar and honeydew using a transparent grid	Beekeeper	After winter, during summer, before winter
	Total surface of combs covered by honey, nectar and honeydew in the bee part of the hive [cm^2]	Estimation of the comb surface containing honey, nectar and honeydew using digital photography followed by image analysis	Beekeeper	After winter, during summer, before winter
Chemical control contamination in bee bread	Total concentration of molecule in bee bread [µg/kg]	Multiresidue analysis	Sampling by beekeeper. Analysis by specialised laboratory	During summer, before winter
Chemical control contamination in wax	Total concentration of molecule in wax [µg/kg]	Multiresidue analysis	Sampling by beekeeper. Analysis by specialised laboratory	During summer, before winter

n: number; ppb: parts per billion.
The variables and methods most suitable for implementation in field surveys across the EU are highlighted in green.
(a): After winter: e.g. 1–2 weeks after bees start foraging, but before first big nectar flow; during summer: active season; before winter: when the colony is preparing for winter (see Section 2.2.2). However, it is clear that more frequent data collection will enrich the data set and is recommended whenever possible. The exact timing of the measurements has to be defined based on the objectives of the field survey (see 'sampling frame' in Section 5).
(b): More detailed info on the quantity of honey in the super is proved in Appendix B, Table B.9; it is mainly done when harvesting honey, which is considered an action related to colony outputs.

3.2.4. Behaviour and physiology of the bees

3.2.4.1. Identification of indicators related to behaviour and physiology of the bees (TOR2)

Honeybees live together in a colony that is a superorganism (Moritz and Fuchs, 1998; Hölldobler and Wilson, 2009), where the many individuals, whose division of labour is highly specialised, function together as a unit (Seeley, 1989). The organisation of work within colonies is both caste-related (workers, queen, drones) and age-related (eggs, larvae, pupae and adults) and is often referred to as 'temporal polyethism'. The temporal caste system through which worker bees progress consists

primarily of cell cleaners, nurses, comb builders, food handlers, foragers and winter bees (Seeley, 1982; Johnson, 2010), although smaller subsets of worker bees will also engage in more specialised tasks like guarding or undertaking (Seeley, 1995). Cleaners are newly emerged bees, but do not comprise a critical functional component of the colony because older bees may also clean cells. Bees aged 4–12 days are called nurses and mainly take care of the brood ('nursing'; e.g. brood feeding and cell capping). These bees may also care for the queen by forming a retinue[19] around her (reviewed in Winston, 1991 and Seeley, 1995). Middle-aged bees (12–21 days) perform multiple tasks including nest building and maintenance, hygienic behaviour, nectar/water receiving and processing, guarding the nest entrance, undertaking and construction of replacement queen cells (Seeley, 1995; Trumbo et al., 1997; Arathi et al., 2000; Breed et al., 2002; Johnson, 2010). After around 20 days, bees usually no longer engage in within-nest tasks and begin to forage (Seeley, 1995). These bees transfer resources from the environment to the hive. Pollen and nectar collection make up most of the foraging activity, except during warm periods when water foraging is also needed for evaporative cooling (Kühnholz and Seeley, 1997) and when the colony requires the collection of resin for the making of propolis. Winter bees are a subpopulation of workers that are responsible for carrying the colony through the winter by forming a thermoregulation cluster, which protects the queen until the re-initiating of brood rearing in late winter/early spring (applicable to temperate climates; Döke et al., 2015). Most hives, with the exception of those that are treated with oxalic acid (Al Toufailia et al., 2015), are not inspected in the winter as part of a routine field inspection and therefore, this indicator will not be considered further in this opinion. Reproduction is the main task of the queen and drones according to the annual life cycle. The queen performance is covered by a separate mind map (see Section 3.2.1) that also considers sperm viability (see Appendix B, Table B.1).

An assessment of the health status of a managed honeybee colony should analyse behaviour and physiology because these influence the demography, defence against infectious agents, pests and predators, as well as the outputs of the colony (see Section 3.1.1). The indicators measuring behaviour and physiology were identified using the methodology described in Section 2.1.2. The indicators are presented in Figure 7 (part A for 'common' and part B for 'caste-specific' behaviour and physiology) and detailed descriptions are provided in Appendix B, Table B.4.

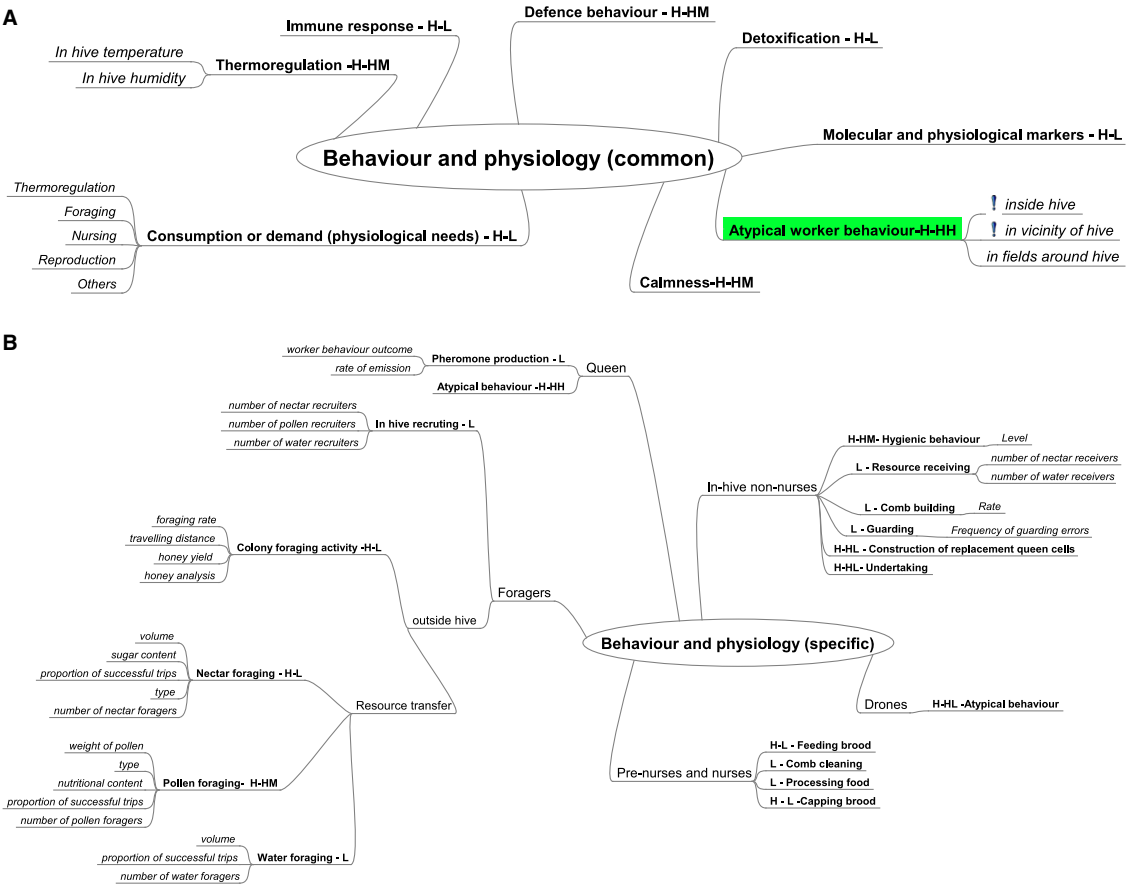
The organisation of work within colonies reflects a compromise between selection for the benefits of division of labour and opposing selection for flexibility in task allocation to facilitate survival of the colony (BR Johnson, 2003, 2010). Several factors, such as genotype, temperature, availability of food resources and photoperiod, mediate physiological changes in individual bees and hence drive their physiological and behavioural development (Huang and Robinson, 1996; Sullivan et al., 2000; Döke et al., 2015). Behaviour refers in this opinion to the fulfilment of a task by a bee. Honeybee behaviours are robust, easily observed and easily recognised (Seeley, 1985, 1995), all of which simplifies the detection of atypical behaviours (e.g. COLOSS BEEBOOK[20]), which is defined here as ectopic or inappropriate given the context/caste of bee: detectable signs of colony illness are often manifested in atypical behaviours (see Appendix B for specific examples). However, it should be noted that an adaptive feature of honeybee behaviours – the flexibility in both task allocation and timing of life history events – represents a particular challenge from a risk assessment point of view. For example, if a particular stressor results in higher forager mortality, the colony is able to adapt by 'promoting' nurse-age bees to forage precociously, which masks the effect of the stressor (Henry et al., 2015). This is sometimes called the 'buffer capacity' of the colony. Additionally, the buffer capacity is also relevant in the detection of disease/viral loads and, with both, representing a challenge in early detection of ill health of a colony. The paragraph below briefly describes 'behaviour and physiology' indicators, in particular those with high scores.

Relevance of behaviour and physiology indicators to the bee health status of a colony

Thermoregulation refers to the honeybees' ability to regulate the in-hive environment, specifically the temperature and humidity. This involves the maintenance of a stable temperature (~ 35°C) across the brood chamber whenever brood is being reared (late winter–early autumn). When the outside temperatures are cool, the bees cluster and warm the brood by activating their muscles; when the outside temperature is warm, the workers cool the hive by bringing in water, which is spread on the surface of the comb, and fanning their wings to promote evaporative cooling and to circulate the air. Lastly, during the winter when brood is not actively being reared, the workers clump around the queen

[19] 'Bees in the retinue regulate queen behaviour via the rate at which they feed her and act as messenger bees by spreading the queen's pheromones about the nest'. Sentence taken from Johnson (2010).
[20] http://www.coloss.org/beebook (last accessed 18 December 2015).

and activate their muscles to heat the cluster, trading off the position of individual workers within the cluster, such that bees in the outer layer are never are cooler than 10°C (Fahrenholz et al., 1989; Seeley, 1995). Note that here the focus is on bees in a more temperate central–northern European setting. In a more southern setting, broodless periods may occur during the summer as a response to high heat.



H-HM, indicators with a High link with bee health, High technical feasibility and Medium priority; **H-HL**, indicators with a High link with bee health, High technical feasibility and Low priority; **H-L**, indicators with a High Link with bee health and Low technical feasibility; **L**, indicators with a Low link with bee health; **!**, recommended variable to assess the corresponding indicator.

Figure 7: Mind map of common (A) and caste-specific (B) behaviour and physiology of honeybees – identified indicators and corresponding scores

Calmness on the comb, which can be scored using a four-point scale, is a sign that all is well in the colony (Büchler et al., 2013). However, because this is a subjective measurement and beekeepers often breed for calmness, this indicator is considered less informative about the health status of the colony.

Defence against infectious agents is covered by the honeybees' immune responses (Evans et al., 2006). The development of the immune system evolves during the life cycle of a honeybee, with a decline in immune responsiveness when foraging (Amdam et al., 2005). These physiological defences are complemented by a repertoire of defence behaviours[21] operating at the individual level (e.g. auto-grooming) or pairwise defences (e.g. allo-grooming) (Cremer et al., 2007) or even a genetically determined behavioural defence against diseases (Spivak, 1996; Al Toufailia et al., 2014). Taken together, analysis of these common responses and behaviours could inform about exposure of the colony to stressors.

Pheromones produced by the queen and workers are important in the biology of the colony (Slessor et al., 2005), but there is currently no evidence that pheromone production informs about bee health in a way that is relevant to this opinion. Typical behaviours of prenurses and/or nurses with a link to the health of the colony are feeding brood to developing bees and covering of the brood

[21] The propensity of the workers in a colony to attack.

(capping brood). Processing nectar into honey and, to a lesser extent, also the cleaning of combs by nurses, are typical behaviours of nurses but there is currently no evidence that gathering detailed data on these would inform about the health status of the colony (see Appendix B, Table B.4). Typical behaviours of in-hive non-nurses related to the health status of the colony are detection, uncapping and removal of dead/dying brood (hygienic behaviour, see above), the creation of cells to raise new, virgin queens (construction of replacement queen cells) and removal of the corpses of dead workers from the hive (undertaking). Resource receiving, comb building and guarding[22] are also typical behaviours of in-hive non-nurses, but there is currently no evidence that gathering detailed data on these would inform about the health status of the colony (see Appendix B, Table B.4). The typical behaviours of foragers comprise informing nestmates about a particular food source (recruiting via waggle dance) or collecting/transporting food resources to the hive (foraging). The quantity of in-hive recruiters is regulated by the colony and is highly variable with season, context and location. Therefore, it is considered that this indicator is not reliable as an indicator of colony health. The provision of pollen, nectar and water is crucial for the survival of the colony. However, water foraging is not a reliable or useful indicator of colony health due to the large variation in foraged water volumes (Seeley, 1995; Kühnholz and Seeley, 1997). Foraging can be decreased by effects of pesticides, infectious agents, pests, or other factors affecting flight and/or orientation performance of foragers (Seeley, 1995; Delaplane et al., 2013a; Yang et al., 2008; Henry et al., 2012; Schneider et al., 2012; Pilling et al., 2013; Riddell Pearce et al., 2013; Ferrari, 2014; Wolf et al., 2014). Therefore, changes in foraging rate may indicate a problem, although the indication is unspecified.

Any atypical (ectopic or inappropriate) behaviour of workers, the queen or drones usually not seen within a given context could be considered as one of the first signs of ill health of the colony, particularly inside the hive and in the vicinity of the hive. For instance, viral infections can lead to crawling of bees in the hive or near the entrance (Ribière et al., 2002). The presence of the queen outside the hive during non-swarming/non-mating flight times is abnormal and indicates a problem. Also the absence of colony foraging during a period with favourable conditions (e.g. warm, sunny days with available forage) is considered an atypical behaviour.

Technical feasibility and priority to include behaviour and physiology indicators relevant to bee health status in field surveys

Overall colony foraging activity is considered a key indicator to describe behaviour given its high relevance in relation to the health status of the colony. For example, a decrease in foraging behaviour and foraging behaviour failure may be due to the sublethal effects of pesticides (Henry et al., 2012; Schneider et al., 2012). However, a common way to study colony-level foraging, which is to monitor manually its foraging rate with counters, will be very difficult to harmonise in the field: colony foraging activity is highly dependent on the environmental conditions, on the food resources available in the RPU, on the time of year and time of day, on the available sunlight, and on the food stock present in the hive. All of this renders a foraging rate per hive that is impossible to compare in a meaningful way with foraging rates across the Member States. Although automatic tools, such as scanners (counters) and radio frequency identification (RFID), provide more precise results on foraging rates and individual foraging behaviour and homing success, it is impractical to consider using these tools routinely in field surveys because the equipment is costly and specialised (Scheiner et al., 2013).

There are methods that could be used to indirectly measure the colony foraging activity over a longer period. Previously, the honey yield (e.g. the amount of honey present in the super and/or harvested by the beekeeper) has been used as a proxy of the colony foraging activity, such that if a sufficient honey yield is collected per period, then one knows that the colony foraging behaviour is proceeding normally. During the workshop, it was mentioned that this process has been used in the bee monitoring programme in Germany[23]. It can be linked to the Section 3.4 'colony outputs' of this paper. Additionally, palynological analysis of honey and pollen (see below) could also be used to identify the botanic origin of the honey, which provides a second proxy of foraging behaviour by indicating on what plants the foragers collected. In other words, if a particular plant is found to be represented in the honey, then one can consider that the colony was foraging normally during that bloom time. However, palynological analysis is specialised and costly and therefore not realistic for inclusion in a large-scale field survey. Lastly, it should be noted that if foraging behaviour is absent when it should be present, then this could be noted as an abnormal behaviour (see below).

[22] Inspecting incoming bees and exclude non-nestmates.
[23] http://www.fisaonline.de/index.php?lang=en&act=research_prog&rp_id=150 (last accessed 8 June 2016).

Within overall foraging, pollen foraging analysis, that is, a particular look at the types of pollen returned in the corbiculae of the pollen foragers, could also be included in a field survey. Here, one analyses the botanic origin of the pollen balls because some plants' pollens are of lower quality (i.e. lower nutritional quality) than others and both pollen type and quantity affect bee health by influencing development, survival and defence responses (Brodschneider and Crailsheim, 2010; Di Pasquale et al., 2013). The beekeeper can take the samples and deliver these to a laboratory for pollen analysis. Likewise, nectar foraging analysis, that is, a particular look at the types of nectar returned in the crop of nectar foragers, could also be included in a field survey. Here, one analyses the botanic origin of the nectar. However, for both, implementation of pollen or nectar foraging analysis is restricted to research activities because it requires sampling of the crop content or pollen baskets of returning foragers and the analysis, which is specialised and costly. Also, the quantification of in-hive recruiters has a low technical feasibility for regular (non-observational) hives (see Appendix B, Table B.4).

Many beekeepers already check explicit atypical worker behaviour inside and around the hive during routine hive inspections because this indicator is considered to alert impaired health. A worker behavioural catalogue has been created by Scheiner et al. (2013) (Appendix F) and could be used as a checklist when assessing the appearance of atypical worker behaviour. Some atypical behaviour of workers may, for instance, include running quickly over the comb for periods (Butler, 2005), trembling (not as part of the tremble dance) (Ribière et al., 2002), failing to cap old larvae (Butler, 2005) shaking or walking around on the ground outside the entrance for long periods of time (Ribière et al., 2002, 2010). Atypical behaviour by the queen is recommended to be included in a field survey when the beekeeper would spend 15 s to 1 min to check, for instance, if the queen runs around on the comb, or lays eggs inappropriately, such as more than one per cell or is unexpectedly laying eggs considering time of the year and bee race (Seeley, 1995; BeeNet, 2013). It should be noted that only explicit atypical behaviour would be detected and that validation of this method is still required before its large scale use in the field. Assessing the atypical behaviour of drones is considered less informative to the health status of the colony compared with assessing the atypical behaviour of workers and/or the queen. Overall, the ability to recognise atypical behaviours depends on the experience of the beekeeper; however, because the normal behaviours are so robust and stereotyped, a beekeeper usually becomes skilled at identifying them quickly.

The freeze-killed brood (FKB) assay could be used to assess the hygienic behaviour of honeybees but requires opening of the hives on successive days and the sacrifice of brood several times per year (Bigio et al., 2013). Defence behaviour could be analysed by counting the number of stings per time unit (e.g. on a flag) (Hunt, 2007; Hatjina et al., 2014b). However, implementation of these three methods by beekeepers in a field survey would require dedicated training to facilitate harmonised data collection. Observation and analysis of the behaviour of construction of replacement queen cells and undertaking have a medium technical feasibility: although it is possible to observe the behaviours, it is less likely once the hive has been opened. This difficulty is combined with the fact that the behaviours are considered to be less informative than the indicators mentioned above (note that actual queen replacement is covered in Section 3.2.1 'Queen presence and performance'). The indicators detoxification, immune response, feeding brood and capping brood have a low technical feasibility for implementation in field surveys.

Molecular and physiological markers provide unique opportunities to investigate bee physiology and health. For example, molecular markers indicating an immune response may demonstrate that the colony has been exposed to an infection (Evans and Lopez, 2004; Huang et al., 2012) or even can be used to predict colony health status (Dainat et al., 2012a; Steinmann et al., 2015). Certain gene expression patterns are linked with exposure to broad ranges of stressors, from pesticides and pests (Gregorc et al., 2012) to acaricides (Boncristiani et al., 2012). The microbial balance, within both the individual and the hive community, including the stored bee products, is becoming an increasingly important area of consideration as it relates to bee health (K. Anderson et al., 2011; Corby-Harris et al., 2014). There is evidence that beneficial, physiological systems within the honeybee, such as the immune or detoxification systems, can be affected by chemical (e.g. pesticide; Gregorc et al., 2012; Di Prisco et al., 2013; du Rand et al., 2015) and seasonal stressors (Steinmann et al., 2015). However, molecular and physiological markers are often unspecified and may not give target information about a specific health problem. Additionally, such sampling and analysis would require specialised training and expensive laboratory tests. As such, they were given a low feasibility within the scope of a field survey outside a research setting.

3.2.4.2. Methods and tools to measure indicators related to behaviour of the bees (TOR3)

Table 7 provides an overview of different variables and methods to measure the indicators 'colony foraging activity' and 'atypical behaviour'. All the methods can be implemented by a beekeeper during

every routine inspection of a hive. It is recommended to collect data on these indicators at least three times a year: after wintering, during the active beekeeping season (summer) and before wintering. If possible, a fourth check to include midspring would be beneficial, as pesticide use is highest during seeding times. However, it is clear that more frequent data collection will enrich the data set and is recommended whenever possible. The exact timing of the measurements has to be defined based on the objectives of the field survey. The text below compares the variables and corresponding methods per indicator and suggests which variable/method is considered most suitable for implementation in field surveys. Detailed information on the suggested methods is provided in Appendix C (Tables C.21–C.23), aiming to further facilitate their harmonisation across the Member States.

Atypical worker behaviour

Individual behavioural anomalies may result from many potential causes and might indicate a problem at the colony level. Atypical worker behaviour can be assessed inside the hive, in the vicinity of the hive or in the fields around the hive. Inside the hive, the recommended method to assess the level of atypical behaviour of the workers is by checking through the hive combs and carefully observing adult bees' activities. Normal worker behaviours are robust and stereotyped and for this reason it is assumed that atypical behaviours will easily be recognised by experienced beekeepers (see Appendix F for more details). The creation of a video library on abnormal worker behaviour might help in harmonising its detection and reporting. Each comb side should be observed during approximately 15 s to 1 min and should be carried out every time the hive is opened for routine inspection.

To assess the level of atypical worker's behaviour in the vicinity of the hive it is recommended to carefully observe the hive entrance plus immediate surroundings (i.e. within a 2 m radius) and check for the presence of adult bees engaged in atypical behaviours. The precision of the assessments in the hive or in the vicinity of the hive will depend precisely on the beekeeper ability to recognise atypical behaviours. It can be done by a beekeeper whenever they walk near the hive. It is not feasible to assess atypical behaviour in the fields around the hive in a standardised way across Europe and for this reason this method was not considered further.

Table 7: Measurement of the selected indicator on behaviour and physiology

Indicator	Variable [unit]	Method
Atypical worker behaviour	Atypical worker behaviour inside the hive [Y/N]	Visual identification of atypical worker behaviour inside the hive
	Atypical worker behaviour in the vicinity of the hive [Y/N]	Visual identification of atypical worker behaviour outside the hive

The variables and methods most suitable for implementation in field surveys across the EU are highlighted in green. They can all be performed by beekeepers, preferably at least three times a year: after winter (e.g. 1–2 weeks after bees start foraging, but before first big nectar flow), during summer (active season) and before winter (when the colony is preparing for winter) (see Section 2.2.2). However, it is clear that more frequent data collection will enrich the data set and is recommended whenever possible. The exact timing of the measurements has to be defined based on the objectives of the field survey (see 'sampling frame' in Section 5). Details on these methods are given in Tables C.21–C.23.

3.2.5. Disease, infection and infestation

A honeybee showing clinical signs is considered to be diseased. Infection refers to the invasion and multiplication of microorganisms, such as bacteria, viruses, and parasites, that are not normally present within a honeybee. Infestation, on the other hand, refers to the external invasion or colonisation of honeybees or their immediate surroundings by arthropods, which may cause disease or are potential vectors of infectious agents.

3.2.5.1. Identification of indicators and methods related to disease (TOR2 and TOR3)

Relevance of disease indicators to the bee health status of a colony

An assessment of the health status of a managed honeybee colony should also analyse disease because this influences its overall health condition. The indicators 'clinical signs' and 'causative agents' are considered to describe disease of a colony and were identified using the methodology described in Section 2.1.2. The indicators are presented in Figure 8 and detailed descriptions are provided in Appendix B, Table B.4. Only the diseases caused by biological agents are considered in this section. Conditions caused by non-organic agents are covered in Section 3.2.3 'in-hive products'.

H-HH, indicators with a High link with bee health, High technical feasibility and High priority; **H-HM**, indicators with a High link with bee health, High technical feasibility and Medium priority. In practice, the determination of the causative agent is subsequent to detection of clinical signs. The score H-HH is highlighted in green as the indicators with this score are taken forward in TOR3, whereas the other indicators not.

Figure 8: Mind map related to disease – identified indicators and corresponding scores

Technical feasibility and priority to include disease indicators relevant to bee health status in field surveys

Visual inspection of a colony will determine the presence or absence of clinical signs in a colony (Figure 8, Table 8). Checking for the presence of clinical signs can be performed by an experienced beekeeper, by carefully observing worker bees and brood (see Appendix D for more details).

Reporting of clinical signs is suggested to be always included in a field survey because clinical signs are one of the major indications of the colony health status. In order to ensure a homogenised inspection of clinical signs, beekeepers should be trained appropriately to recognise them and implement this every time they visually inspect a hive. Observation of clinical signs will be performed at every hive inspection, although, depending on the disease, some of them will be observed only at certain times of the year. The sensitivity will depend on the severity of disease and on the observer's ability to recognise the clinical signs. The specificity is considered to be low because similar clinical signs can be caused by several agents and/or chemical control contaminants, such as pesticides (see Appendix D). Apart from direct observation of clinical signs, diagnostic methods are available for most infectious agents and pests, therefore, it is recommended to identify the causative agent in as many cases as possible. All positive and negative laboratory results should be reported, specifying the test method used.

A suspected disease case occurs when clinical signs are present without identification of the infectious agent or pest. The absence of clinical signs is interpreted as 'no disease detected' (when no clinical signs are observed and no analysis is done to detect infection and/or infestation), 'presence of infection' (when no clinical signs are observed and one or more infectious agent(s) or pest(s) are identified) or 'absence of infection' (when no clinical signs are observed and analysis did not identify the presence of specified infectious agent(s) and/or pest(s)). Identification of an infectious agent or a pest in an endemic area (e.g. *V. destructor* and *Paenibacillus* larvae) together with the presence of clinical signs could be seen as a confirmed disease case. Identification of a pest in a non-endemic area (e.g. *Aethina tumida* or *Tropilaelaps* spp.) must be confirmed by laboratory analyses[24] and will require subsequent notification.

Table 8: Measurement of selected indicators on disease

Indicator	Variable [unit]	Method
Clinical signs	Clinical sign [Y/N] (see Appendix D)	Visual inspection

The method suggested for implementation in field surveys is highlighted in green. It can all be performed by beekeepers, preferably at least three times a year: after winter (e.g. 1–2 weeks after bees start foraging, but before first big nectar flow), during summer (active season) and before winter (when the colony is preparing for winter) (see Section 2.2.2). However, it is clear that more frequent data collection will enrich the data set and is recommended whenever possible. The exact timing of the measurements has to be defined based on the objectives of the field survey (see 'sampling frame' in Section 5). Details on these methods are given in Table C.24.

3.2.5.2. Identification of indicators related to infection or infestation (TOR2)

Relevance of infection or infestation to the health status of the colony

It is possible that a managed honeybee colony does not show clinical signs but is infected or infested with an infectious agent or pest, respectively. The impact of infection or infestation on the health status of the colony is dependent on the characteristics of the infectious agent or pest and on the genetics of the bees and their natural resistance to the pathogens (e.g. Spivak and Gilliam, 1998; Danka et al., 2012; Khongphinitbunjong et al., 2012; Büchler et al., 2010; Hamiduzzaman et al., 2015; Strachecka et al., 2015; Liu et al., 2016; Strauss et al., 2016). In this opinion, the capacity to induce

[24] This is particularly true for exotic pests, that local beekeepers and veterinarians are not used to see.

disease was considered a prerequisite for an infectious agent or pest to be scored highly relevant to the health status of the colony. Therefore, low pathogenic agents and commensals should not be assessed in field surveys when no disease is observed. The mite *Varroa destructor* is a parasite of adult honeybees and their brood and the course of this parasitism is usually lethal (World Organisation for Animal Health, 2008a,b,c). However, the role of *V. destructor* alone is not clear because the mite is often a carrier and amplifier of viruses, in particular deformed wing virus (DWV; Martin et al., 2001; World Organisation for Animal Health (OIE), 2008a,b,c; Genersch and Aubert, 2010; Martin et al., 2012; Nazzi et al., 2012). *Paenibacillus* larvae infect bee brood, and may lead to American Foulbrood (AFB)which is known to cause high mortality in larvae after their cells are sealed (World Organisation for Animal Health, 2013). *Melissococcus plutonius* infects honeybee brood, leading to European Foulbrood (EFB) and larvae usually die 1–2 days before being sealed in their cells (World Organisation for Animal Health (OIE), 2008a,b,c; Forsgren et al., 2013). The mite *Tropilaelaps* spp. feeds on bee larvae and pupae, leading to brood malformation; however, these mites are not considered relevant when assessing the health status of a honeybee colony since *Tropilaelaps* spp. are exotic to Europe.[25] The coleopteran *Aethina tumida*, known as the small hive beetle (SHB), feeds on honeybee brood, honey and pollen, and may lead to brood death and honey fermentation (World Organisation for Animal Health, 2013). The SHB was exotic to Europe until September 2014, when it was detected in Calabria (Italy) (Mutinelli et al., 2014; Palmeri et al., 2015). The beetle was also detected in the same area in October 2015, as well as in April and May 2016.[26] Although the SHB has been detected in only one Member State to date, it is possible spread throughout Europe would represent an additional health hazard for honeybees. Natural spread of the beetle is slow but movement of an infested hive could spread SHB rapidly over large distances (EFSA, 2015). *Nosema* disease caused by *Nosema apis* has been reported as a serious disease of honeybees in temperate climates (Fries, 1993). *Nosema ceranae* has been implicated in colony population depletion (Higes et al., 2006, 2008), although the role of *N. ceranae* itself is not clear. Some reports of experimental studies describe an interaction between *N. ceranae* and other stressors (e.g. chronic bee paralysis virus (CBPV), black queen cell virus (BQCV) or the neonicotinoid imidacloprid) can lead to elevating honeybee mortality (Alaux et al., 2010; Toplak et al., 2013; Doublet et al., 2015).

DWV, CBPV, acute bee paralysis virus (ABPV) and Sacbrood Virus (SBV) have been linked with clinical signs in honeybee colonies, whereas it is less clear if other viruses could induce disease (see Appendix B, Table B.5). There are many other infectious agents and pests that could affect honeybee health although they have not been considered as major factors impacting honeybee health because: (i) it is not clear if the induced infections will cause disease, and/or (ii) they are not known to be present throughout Europe, and/or (iii) it is known that these agents induce disease but disease impact on honeybee health has been considered to be low (e.g. *Ascospahaera apis*, *Acarapis woodi*) (see Figure 9 and Appendix B, Table B.5).

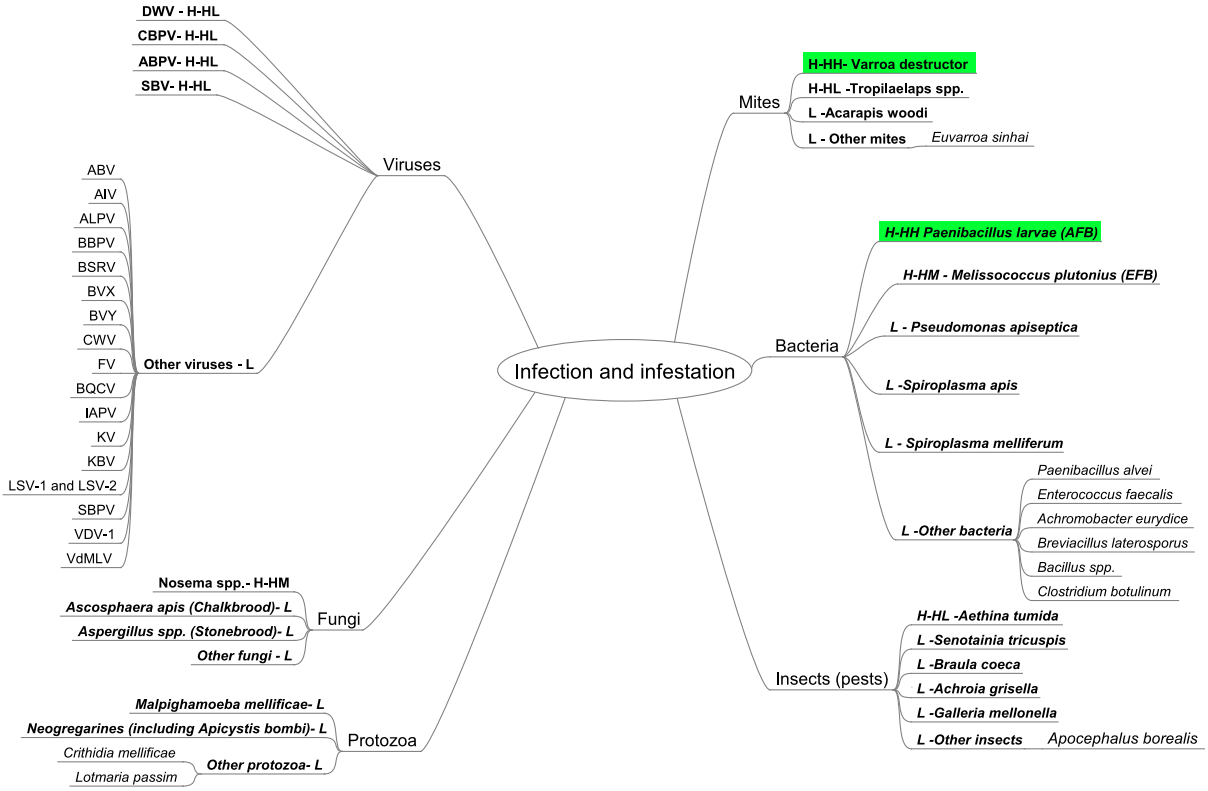
Technical feasibility and priority to include infection or infestation relevant to bee health status in field surveys

The technical feasibility and priority to assess infection or infestation by an infectious agent or pest in a field survey are scored as described in Section 2.2.1 and details are provided in Appendix B, Table B.5. Details on the recommended methods can be found in Appendix C, Tables C.25–C.27.

Analysing the presence of *V. destructor* in a colony is already performed by many beekeepers in many various ways and is considered of high priority even in the absence of clinical signs because it is widespread in Europe and can be an important factor in colony mortality (Le Conte et al., 2010). The number of *Varroa* mites in the colony has to be assessed in order to evaluate the parasitic pressure, because below a certain threshold the infestation level does not affect the colony health seriously (see, for example, Giacobino et al., 2015). It is required to report if brood is present in the hive and if a treatment has been applied before the inspection (see Section 3.3.3).

Several laboratory techniques are available for detecting infection by *Paenibacillus larvae*. The bacteria are widespread in Europe, highly contagious and the spores produced by *P. larvae* are extremely tenacious (Morse and Flottum, 1997; Genersch, 2010). AFB cases are notifiable in the EU.[27]

[25] Findings of *Tropilaelaps* spp. are notifiable in the EU (Council Directive 82/894/EEC and Council Directive 92/65/EEC as last amended).
[26] http://www.izsvenezie.it/aethina-tumida-in-italia/ (last accessed 24 May 2016).
[27] Commission Directive 92/65/EEC.



H-HH, indicators with a High link with bee health, High technical feasibility and High priority; **H-HM**, indicators with a High link with bee health, High technical feasibility and Medium priority; **H-HL**, indicators with a High link with bee health, High technical feasibility and Low priority; **H-L**, indicators with a High Link with bee health and Low technical feasibility; **L**, indicators with a Low link with bee health.. The score H-HH is highlighted in green as the indicators with this score are taken forward in TOR3, whereas the other indicators not.

Figure 9: Mind map of infection or infestation – identified indicators and corresponding scores

Melissococcus plutonius infection can lead to colony collapse but clinical signs were reported in only 5 of 15 Member States involved in the EPILOBEE study between 2012 and 2014 (Laurent et al., 2015 and see Appendix B, Table B.5). Therefore, when no clinical signs are detected, it is recommended to assess the presence of *M. plutonius* only in hives that are located in areas where EFB has been reported in recent years. As for *P. larvae*, several laboratory techniques are available for detecting infection caused by *M. plutonius*.

Nosemosis, based on the clinical signs observed in the presence of type A nosemosis (*N. apis*), has been reported in 10 of 15 Member States involved in the EPILOBEE study between 2012 and 2014 (Laurent et al., 2015). When no clinical signs are detected, it is recommended to assess the presence of *Nosema* spp. only in hives that are located in areas where nosemosis has been reported in recent years. If *Nosema* spp. are detected, it is recommended to quantify the infection level, although interpretation of this quantification is not easy. Indeed, no threshold, associated with the observation of clinical signs and presence of the disease, has been commonly adopted. Nevertheless, *Nosema* spp. quantification gives information on the infection level, which might be very useful, especially in cases of coexposure (with pesticides, for example). The latter might help to unravel why *Nosema* spp. are causing mortality in southern, but not northern, Europe.

As mentioned above, *A. tumida* is only detected in one Member State at the moment. Detection of SHB could be included in field surveys, in particular in affected areas. Positive findings on the EU territory must be reported since it is a notifiable disease.[28]

There are polymerase chain reaction (PCR) methods available for many bee viruses (de Miranda, 2008) although a few of them are validated to date. Several papers describe the involvement of bee viruses in a multifactorial context of bee mortality. The DWV is closely associated with *V. destructor* (Ribière et al., 2008) and is considered one of the most implicated infectious agents of honeybee

[28] Council Directive 82/894/EEC and Council Directive 92/65/EEC as last amended.

decline from various studies conducted in several Member States (Bacandritsos et al., 2010; Genersch et al., 2010; Budge et al., 2015). CBPV infections have been associated with the collapse of the colony (Allen and Ball, 1996; Ball and Bailey, 1997; Ribière et al., 2010). Clinical prevalence of CBPV has been reported in 14 out of 17 Member States during the three visits of EPILOBEE during 2013–2014 (Laurent et al., 2015). ABPV has been detected in several Member States and is more common in Europe than other closely related viruses from the Family Dicistroviridae, like Israeli acute paralysis virus (IAPV) and Kashmir bee virus (KBV) (Cox-Foster et al., 2007; see as reviews Allen and Ball, 1996; de Miranda et al., 2010; Ribière et al., 2008). ABPV commonly occurs at low levels in apparently healthy bee colonies. However, several studies reported that the virus can be a major cause of mortality in colonies infected with *V. destructor* in several Member States (see as reviews Allen and Ball, 1996; Ribière et al., 2008; de Miranda et al., 2010). SBV has been detected in high quantities in dead adult bees in colonies infected with *V. destructor* in Poland and Germany (Bailey et al., 1964; Ball, 1999). Taken together, detection of DWV, CBPV, ABPV and/or SBV in a honeybee colony without clinical signs could be included in some field surveys, in particular when investigating their role in a multifactorial context.

In summary, it is recommended to assess systematically in field surveys the infection and infestation status of a honeybee colony regarding *P. larvae* (detection of agent) and *Varroa* (quantification of mites). When clinical signs are observed, it is recommended to identify the causative agent when feasible (see Section on 'disease' above).

3.2.5.3. Methods and tools to measure indicators related to infection or infestation (TOR3)

The text below compares the methods to assess the indicators '*Varroa* infestation' and '*P. larvae* infection', and suggests which ones are considered most suitable for implementation in field surveys. This information is summarised in Table 9. Detailed information on the suggested methods is provided in Appendix C (Tables C.24–C.27), aiming to further facilitate their harmonisation across the Member States.

Varroa infestation

Varroa infestation level can be assessed at a colony level, at adult bee's level or by directly inspecting drone brood (Table 10). For the purpose of harmonisation, it is recommended to assess this indicator by sampling adult bees and sending them to a laboratory for mite counting. Determining the level of *Varroa* infestation in adult bees can be performed by submerging adult bees in alcohol (75%) to dislodge the mites (World Organisation for Animal Health (OIE), 2008a,b,c). The use of soapy water is an effective and economical alternative to washing with alcohol for mite detection on adult bees (Dietemann et al., 2013; Dobrynin et al., 2013). However, this method may decrease the accuracy of the outcome due to possible variability in the temperature of water, soap type and concentration, so it is less suited for harmonisation than the alcohol method. Using powdered sugar to dislodge the mites does not kill the bees and is an environmentally friendly technique, but is considered less precise than the alcohol/soapy water methods (Dobrynin et al., 2013; Flores et al., 2015).

Varroa infestation level can also be assessed by inspecting large amounts of brood (World Organisation for Animal Health (OIE), 2008a,b,c; Dietemann et al., 2013). This method is not suitable for application in extensive field surveys because it can only be performed during the presence of brood and is resource consuming (Dobrynin et al., 2013). Moreover, it does not allow determination of the level of infestation during periods when the colonies are usually treated against *Varroa* (autumn and winter). *Varroa* infestation level can also be assessed by using sticky boards placed in the bottom of the hive to trap the mites after natural fall. The accuracy of this method is difficult to determine because it is largely influenced by the amount of emerging infested brood (Dietemann et al., 2013). However during the brood-rearing period, it is generally considered a good indicator of colony infestation (Branco et al., 2006; Flores et al., 2015). Traps could also be applied after treatment (World Organisation for Animal Health (OIE), 2008a,b,c). Chemical treatment must be applied carefully according to the manufacturer's recommendations, in order to avoid contaminating honey that will be harvested for human consumption.

The timing of assessing the *Varroa* infestation level depends on the method used (see Table 10 and Appendix C.1.5). For instance, it is expected that *Varroa* mite prevalence is highest at the end of beekeeping season in untreated colonies (September/October in Northern Hemisphere climate; World Organisation for Animal Health (OIE), 2008a,b,c). If a treatment is applied in the end of summer, assessment should be performed just before winter. If a treatment has been performed this should be

reported in a standardised way, together with the date and the type of treatment (see Section 3.3.3), to allow better understanding of the mite population dynamics in the colony.

P. larvae *infection*

In *P. larvae*, two cases have to be distinguished: detection and identification of the bacteria from a colony suspected to suffer from AFB (showing clinical signs), and detection and identification of the bacteria from a colony without any clinical signs (e.g. in the framework of an AFB monitoring/prevention programme). Depending on the context, the matrix of choice and the recommended methods will vary. In the case of AFB suspicion, there are several laboratory techniques to identify *P. larvae.* PCR is highly sensitive, allows confirmation of the species and it is routinely applied in several laboratories across Europe. Details on this method can be found in Table C.27 (Appendix C). In the case of detection and identification of the bacteria in a colony without clinical signs, the recommended method to be used is also PCR, which is the most sensitive method. However, the matrix of choice would not be larvae, but honey, adult bees or debris (Nordstrom and Fries, 1995; Ritter, 2003; Pernal and Melathopoulos, 2006; Gende et al., 2011; Forsgren and Laugen, 2014).

Identification of spores of *P. larvae* in brood through microscopy is a less preferred method because it has low specificity (the spores can be confused with *P. alvei*, a secondary agent of EFB) and is not conclusive (the confirmation of the agent requires further steps). The isolation and identification of *P. larvae* through culture methods can be done using several matrices (honey, wax, pollen, bees, larvae), but is also not recommended because it has low sensitivity (low germination of *P. larvae* spores in culture medium) and it is not conclusive (the confirmation of the agent requires further steps). Mass spectrometry is not commonly used in European laboratories and biochemical methods are not specific to *P. larvae*. Hence these methods are also not recommended for application in extensive field surveys. Antibody-based techniques can be used for the identification of bacterial colonies resulting from a culturing step or for direct examination of larval remains (World Organisation for Animal Health, 2014). However, they are resource consuming and therefore not preferred. A lateral (antibody-based) flow device for rapid confirmation of AFB has been commercialised (Blacquière and van der Steen, 2006; World Organisation for Animal Health, 2016) and is, for instance, currently used in England and Wales to diagnose AFB in the field as part of the 'bee health programme' when diseased larvae are observed (Wilkins et al., 2007).

Table 9: Measurement of selected indicators on infection and infestation

Indicator	Variable [unit]	Method	Implementation	Timing[(a)]
Varroa infestation	*Varroa* infestation level in the hive [number of mites collected per day]	Capturing *Varroa* mites using a sticky trap natural fall	Beekeeper	After winter, before winter
		Capturing *Varroa* mites using a sticky trap after treatment	Beekeeper	After winter, before winter
	Varroa infestation level on adult bees [number of mites per 100 bees]	Counting the number of mites dislodged with alcohol	Beekeeper – sampling; laboratory – mite counting	Before winter
		Counting the number of mites dislodged with sugar	Beekeeper – sampling; laboratory – mite counting	Before winter
		Counting the number of mites dislodged with soapy water	Beekeeper – sampling; laboratory – mite counting	Before winter
	Varroa infestation level in drone brood [number of mites per 100 bees]	Visual inspection of drone brood	Beekeeper	After winter, during summer, before winter

Indicator	Variable [unit]	Method	Implementation	Timing[(a)]
P. larvae infection	Presence of *P. larvae* with clinical signs (disease) in the hive [yes/no]	Identification of the species *P. larvae* through PCR (conventional and real-time PCR) on diseased larvae (in the presence of clinical signs)	Beekeeper – sampling; laboratory – analysis	Each time clinical signs are observed
		Lateral flow device test	Beekeeper/inspector	Each time clinical signs are observed
	Presence of *P. larvae* without clinical signs (infection) in the hive [yes/no]	Identification of the species *P. larvae* through PCR (conventional and real-time PCR) on honey/adult bees/ debris (in the absence of clinical signs)	Beekeeper – sampling; laboratory – analysis	After winter, during summer, before winter
	Presence of *P. larvae* with or without clinical signs (infection) in the hive [yes/no]	Identification of spores or bacilli of *P. larvae* through microscopy	Beekeeper – sampling; laboratory – analysis	Each time the identification of *P. larvae* is required. Can also be done after bacterial culture. After winter, during summer, before winter
		Isolation of *P. larvae* and morphological identification of bacterial colonies through culture methods	Beekeeper – sampling; laboratory – analysis	Each time the identification of *P. larvae* is required. After winter, during summer, before winter
		Mass spectrometry	Laboratory	Each time the identification of *P. larvae* is required. After winter, during summer, before winter
		Biochemical tests	Laboratory	Each time the identification of *P. larvae* is required. After winter, during summer, before winter
		Identification of *P. larvae* using antibody-based techniques	Laboratory	After winter, during summer, before winter

PCR: polymerase chain reaction.
The methods most suitable for implementation in field surveys across the EU are highlighted in green.
(a): After winter: e.g. 1–2 weeks after bees start foraging but before first big nectar flow; during summer: active season; before winter: when the colony is preparing for winter (see Section 2.2.2). However, it is clear that more frequent data collection will enrich the data set and is recommended whenever possible. The exact timing of the measurements has to be defined based on the objectives of the field survey (see 'sampling frame' in Section 5).

3.3. External drivers affecting the health status of a managed honeybee colony (TOR2–3)

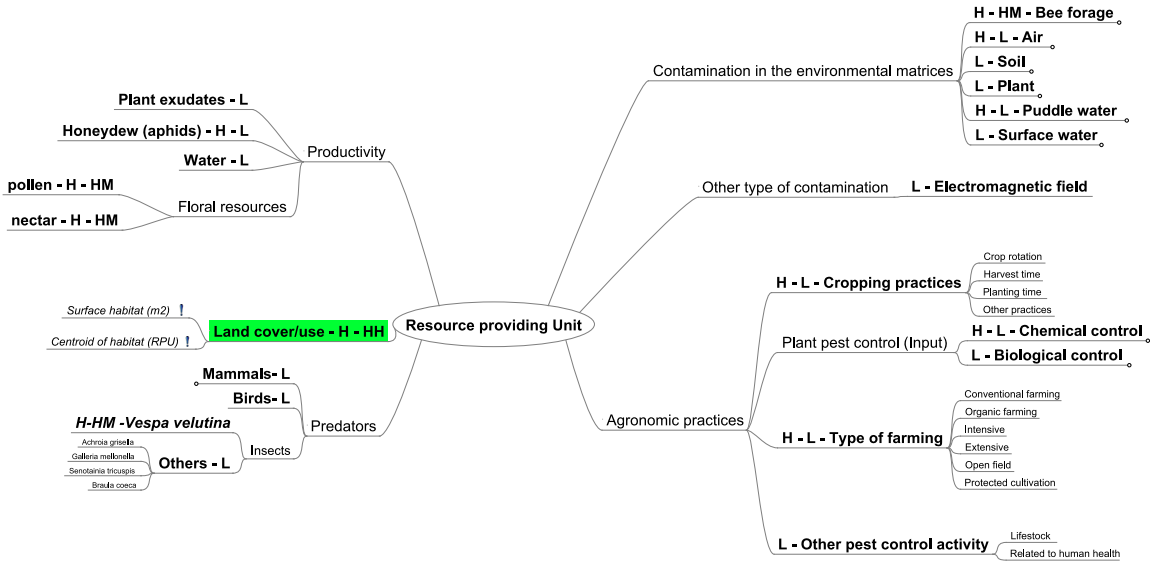
The external drivers are defined as overarching concepts that reflect the multidimensional characteristics of the colony habitat and management. They can only be assessed indirectly.

3.3.1. Resource providing unit (TOR2)

The RPUs adapted from the definition of the service providing unit (Gilioli et al., 2014) and comprises the environmental components or units responsible for the genesis and regulation of the resources for a colony. The shape and the area of the RPU are defined by the foraging distance reached by the honeybees of a given colony in all the possible directions starting from the hive. The simplest assumption is that RPU has a round shape with the centre in the hive; different shapes can be hypothesised according to the characteristics of the landscape (e.g. the presence of large water bodies). The structural (e.g. the position and the dimension of the different crops) and functional (e.g. the productivity in pollen of the different crops in the RPU) characteristics of the RPU provide information on the availability, type, amount and accessibility of the resources. The RPU can be divided

into subunits or patches that are considered homogeneous areas from a resource production point of view (EUNIS, 2007; MNHM and EEA, 2014). The average travelling distances during foraging can be considered as an indicator of the patches (i.e. habitats) quality and the resource availability and depends on the colony size. A 3 km average foraging distance and a 10 km maximum foraging distance from the hive were estimated (Van der Steen, 2015; EFSA, 2016a).

The RPU influences in-hive products; hence information on the RPU is required when interpreting indicators of bee health. The paragraph below briefly describes the 'RPU' indicators, in particular those with high scores.



H-HH, factors with a High relevance to bee health, High technical feasibility and High priority; H-HM, factors with a High relevance to bee health, High technical feasibility and Medium priority; H-HL, factors with a High relevance to bee health, High technical feasibility and Low priority; H-L, factors with a High relevance to bee health and Low technical feasibility; L, factors with a Low relevance to bee health; !, recommended variable to assess the corresponding indicator. The score H-HH is highlighted in green as the factors with this score are taken forward in TOR3, whereas the other factors not.

Figure 10: Mind map resource providing unit – identified factors and corresponding scores

3.3.1.1. Relevance of the RPU factors to the bee health status of a colony

The RPU can be described by factors, such as productivity, land cover, contamination in the environmental matrices and agronomic practices (see Figure 10). Further details are available in Appendix B, Table B.6 where a comprehensive list of RPU factors is presented together with their respective variables.

Land cover/use is a major factor of the RPU as it determines the forage availability in quantity and in quality. Following the definition provided in the Eurostat's Concepts and Definitions Database (Eurostat, 2016), land cover is defined as: 'the observed (bio) physical cover on the earth's surface. It is that which overlays or currently covers the ground. This description enables various biophysical categories to be distinguished – basically, areas of vegetation (trees, bushes, fields, lawns), bare soil (even if this is a lack of cover), hard surfaces (rocks, buildings) and wet areas and bodies of water (sheets of water and watercourses, wetlands). It is said that Land Cover is 'observed'. This means that observation can be made from various 'sources of observation' at different distances between the source and the earth's surface: the human eye, aerial photographs, and satellite sensors'.

Whereas 'the land use is characterised by the arrangements, activities and inputs people undertake in a certain land cover type to produce, change or maintain it' (Fao, 1999).

The land cover/use describes and characterises the foraging area around the colony. The surface of the habitat indicates the relative contribution of different food source and other resources into the RPU.

Kandziora et al. (2013) compared four different land use data sets, spatially explicit, for their practicability as input data for an assessment of ecosystem services in a specific area. The authors concluded that a spatial resolution at crop level is needed to assess provisioning ecosystem services.

In this paper, the land cover/use is considered in terms of habitat and resources (forage) provided to the bees.

Agronomic practices include type of farming, crop practices, plant pest control and other pest control activities. Cropping practices (e.g. crop rotation, grazing/mowing before flowering) may limit the diversity of crops available within the RPU; therefore, it can influence the available forage to the honeybees (AFSSA, 2009; Hooper, 2010). Depending on the timing or the provision of alternative forage sources, the health of the honeybee colony might be affected, in some cases compromising the winter survival of the colony (AFSSA, 2009). Furthermore, crop practices are directly linked to land use; thus, they should be addressed at a crop–temporal scale when assessing the factor 'land cover'.

The most relevant plant pest control method that might affect honeybee colony health is chemical control that consists of the application of a chemical treatment on a crop to reduce the potential negative effects of the pests (de Miranda et al., 2010; EFSA, 2012a, 2013). Many different pesticides (namely insecticides) adversely affect the health of the bees exposed to them. However, the purpose of this opinion is not to provide a list of compounds to take into consideration. Also the intensity of the agricultural interventions on a crop vary depending on the type of farming that is applied in the RPU (e.g. monoculture versus polyculture; intensive versus extensive; organic versus conventional farming).

The pollen, nectar and honeydew productivity of the RPU are essential factors representing the amounts produced at each subunit level (i.e. habitat) of the RPU in unit time (e.g. 7 days). A lack of pollen and/or nectar in the hive affects the honeybee colony health (Dietz, 1975; Di Pasquale et al., 2013). Analysis of the main food sources available within the RPU as well as in the hive may inform on the capacity of the colony to forage, store and consume feed.

In terms of contamination, among the various environmental matrices, contaminated bee forage, air and puddle water are identified in this opinion for consideration in the assessment of colony health. Contamination is defined in terms of the presence of contaminants (e.g. pesticides) in the environmental matrices available in the RPU habitats. Regarding bee forage, the oral uptake of contaminated nectar is a main route of exposure to pesticides (EFSA, 2012a, 2013). If a substantial proportion of the honeybee colony's forage contains high pesticide residues in nectar, this might lead to the death of the colony, whereas contaminated pollen affects larval development and nurse bees (Wu et al., 2011; EFSA, 2012a, 2013). Honeydew is a sugar-rich secretion produced by aphids and scale insects feeding on plant sap. Honeybees collect honeydew and process it into honey called 'honeydew honey' (Maurizio, 1985). Oral uptake of contaminated honeydew can have severe consequences on the colony (Hagenbucher et al., 2014). This is the case, for instance, when insecticides are used on a crop infested by aphids, and could have an adverse effect on the foragers attracted by the crop for collecting honeydew (EFSA, 2013). Air is also mentioned as an important route of exposure, mainly in terms of the drifting of pesticides related to their use on crops in the RPU (Marzaro et al., 2011; Girolami et al., 2012). Moreover, considering that bees prefer to consume water from the puddles (EFSA, 2013) and that the concentration of contaminants in puddle water in the vicinity of treated crops seems to be higher than for surface water in general (EFSA, 2012a), this environmental matrix has been considered as highly relevant for its effect on honeybee colony health.

In regards to predators, the yellow-legged hornet *Vespa velutina*, which was first reported in France in 2005 (Haxaire et al., 2006) and subsequently spread throughout Western Europe (Villemant et al., 2011; Monceau et al., 2014), is of major importance for beekeepers and contributes to colony losses (Monceau et al., 2014). Collection of data on the presence of *Vespa velutina* is recommended in field surveys in affected areas.

Birds, mammals and insects (other than *Vespa velutina*) can have an impact in the colony by either predating the bees, damaging the hive or eating the food stock. They do not induce disease and their survival is not strictly dependant on the honeybee life cycle. Therefore, in this framework, they were considered as external factors. The impact induced by birds, insects or mammals on European beekeeping is not well documented but experts consider that the impact is minor.

3.3.1.2. Technical feasibility and priority to include RPU factors in field surveys

The land cover/use

For characterising and describing the land cover at the EU level, the CORINE Land Cover (CLC, 2006) defined at three levels might be sufficient, whereas for an assessment at a local or Member

State level a fourth level might be needed. Also some crops that provide pollen and/or nectar likely to benefit bees should be addressed specifically (Hooper, 2010). In that context, the availability of resources during the growing season should also be considered. For example, EUNIS (2007), provides this information based on the RPU subunits (habitats), which are defined by the environmental characteristics and often by the dominant plant species. Table 11 provides examples of different land cover/use data sets that could be used depending on the level of resolution and type of information required by the assessment.

To assess the type and quantity of resources used by the hive, different methods should be used and their results combined:

- Land cover/use provides information that describes and characterises the resources that could potentially be visited by the foragers. It is important to mention that honeybees also forage largely on flowers from hedgerows and that these are not always captured by the available land use maps and/or data. Different data sets are available and could be used depending on the objectives of the assessment, but none of them was conceived from a honeybee perspective. The determination of the distance between the hive and a homogeneous unit of the RPU should be based on aerial photo-interpretation or alternatively by accessing databases of land use. Geographical information systems are commonly used to spatially explicit the land cover/use.
- Palynological analysis of the pollen within the nectar in the hive will provide information of the type of flowers the foragers have visited. It is important to mention that during their flight, forager bees might lose some pollen, and therefore, this analysis will not provide a complete picture of the visitation activity.
- Observation of the waggle dance of the forager bees that can share with the other members of the colony information about the direction and distance to patches of flowers yielding nectar and pollen. The exact type of flowers being visited is still difficult to interpret. Analysis of the waggle dance can only be done in a research setting.

Using the complementary results obtained with these three different methods, it should be possible to identify the type of flowers visited over time within the RPU.

Although many factors of the RPU are highly relevant to the health of the honeybee colony and methods exist to measure them, they have not been considered as priorities in data collection during field surveys. This is, for instance, the case in chemical control and contamination of environmental matrices, which despite having a very strong influence on the health status of a honeybee colony, were not retained as a priority considering the high cost of collecting the data, variability in the data across the different habitats where honeybees are present and variability in the data within the EU. However, these factors could be estimated through different methods, such as modelling, and the use of existing databases available at national, regional or local level on trade or pesticides consumption (Eurostat, 2007, 2012). These estimates could be cross-checked with the list of pesticides that have been identified in a hive (see Section 3.2.3 on in-hive products). The level of contamination of the different environmental matrices is directly related to the means of application of the chemical products, the crop to be treated and the timing of the chemical treatments in the fields, the active substance/ingredient and concentration on the crop within the RPU fields. These parameters should be considered when estimating possible exposure of the bees to pesticides, although they are limited to a research setting.

3.3.1.3. Methods and tools to measure factors related to RPU (TOR3)

The Panel recognises that, depending on the spatial and temporal extent of the assessments to be performed, the required level of resolution of the land cover/use is variable. Although different land cover/use databases are available at the EU level (Table 10), they were not conceived and developed to cover the resources foraged by bees and the choice of the most appropriate one depends on the needs of the analyses, the location of the RPU, the spatial resolution of the analyses (local, regional, national, continental), the time scale of the analyses and the availability of existing databases for characterising the RPU.

In principle, the CORINE Land Cover (CLC, 2006) and the EUNIS (2007) classification can be applied to the full extent of the EU-28.[29] Land cover characterisation at the EU level could be limited to

[29] The union reached its current size of 28 member countries with the accession of Croatia on 1 July 2013 (See https://europa.eu/european-union/about-eu/countries_en, last accessed 02 September 2016).

the third level of the CORINE Land Cover classification on a scale of 1:100,000. However, this level does not reflect the detailed resource availability within the RPU. Moreover, this land cover classification across the EU corresponds to 2006 land cover based on satellite imaging and does not provide information on variations in land cover over time (mainly regarding the annual crops). Therefore, as an example, the Panel presents the land cover defined at four different levels (see Appendix C, Table C.28). The first three levels are presented and defined in the CORINE Land Cover nomenclature as follows: 'The CORINE Land Cover is a vector map with a scale of 1:100 000, a minimum cartographic unit (MCU) of 25 ha and a geometric accuracy better than 100 m. It maps homogeneous landscape patterns, i.e. more than 75% of the pattern has the characteristics of a given class from the nomenclature. This nomenclature is a three-level hierarchical classification system and has 44 classes at the third and most detailed level. In order to deal with areas smaller than 25 ha a set of generalisation rules were defined'. For the needs of the analysis of the effect of land cover on the health of the bee colonies it was considered necessary to include an additional fourth level based on the EUNIS (2007) classification. EUNIS (2007) is a comprehensive pan-European system to facilitate the harmonised description and collection of data across Europe through the use of criteria for habitat identification. It is hierarchical and covers all habitat types from natural to artificial, from terrestrial to freshwater and marine. Subsequently, crop species are described (in case of cropped areas), as for example under the EUNIS habitat code I1.1 'Intensive unmixed crops'. This example combining the CORINE Land Cover and the EUNIS classification is presented in Appendix C (Table C.28).

However, the Panel acknowledges that the spatial and time resolution of land cover classification (combining the three levels of CORINE Land Cover and a fourth level based on EUNIS, 2007) should be integrated/implemented by assessing the type of forage (i.e. crops availability) and the forage seasonality (i.e. flower blooming period) in order to better characterise the resource availability within the RPU. Moreover, because 'agronomic practices' might reflect the land use (if addressed at crop-temporal scale), they should be taken into account when assessing the factor 'land cover/use'.

At the EU level, the Land Use and Cover Area Frame Survey (LUCAS, 2012) based on survey data, images and statistical data could also be used. This database has been developed to allow compatibility with other existing land cover/use systems. The data is available at NUTS[30] 1, 2 and 3 levels in the EU for a very wide number of crops. However, it provides data based on the surveys and does not provide with exactitude the description of all the crops in the RPU. This database is updated every 3 years. LUCAS survey land use classification 2012 contains 14 main classes among which there are 48 different categories of cropland and forests. For detailed information consult the technical reference of the land use and land cover classification (LUCAS, 2012; the 2015 version is pending validation). Information on the LUCAS database and the Land Use Database of the Netherlands (LGN7, 2013), are presented in Table 10.

Regarding the flower resources available in the RPU, honeybees cannot be sustained from crops alone and require pollen and nectar resources from a variety of habitats within the landscape. The sum availability of these resources within the landscape can, therefore, have a substantial effect on colony size and productivity. In particular, although the floral resources are available in the RPU, if the in-hive products do not meet the needs of the colony, this might indicate a poor health status. For instance, Baude et al. (2016) provide estimates of these resources from more than 200 plant species in the UK, enabling estimation of the availability of pollen and nectar resources at the landscape scale without new primary data collection. However, this database looks only at plants in the UK and does not consider wider flora; neither does it consider the effects of climate on resource production. Therefore, the Panel acknowledges that new primary data will be required to expand this database across the EU.

Table 11 provides information on the spatial and temporal resolution of different databases on land use that could be used. Some of these databases have been developed at the Member State level, others at the EU level.

As mentioned above, in order to identify the type of flowers visited by the forager bees over time within the RPU, it is suggested to analyse not only the results obtained by a characterisation and description of the flower and resource availability in the RPU, but also to perform palynological analysis of the nectar in the hive and, if possible, to observe and interpret the waggle dance.

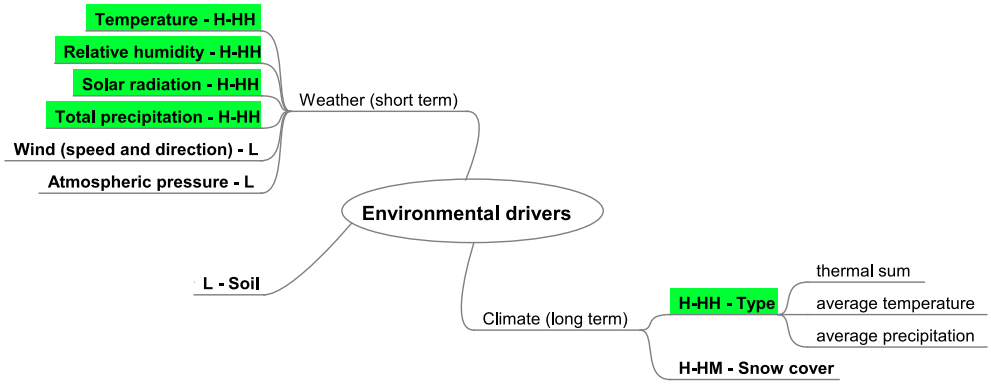
[30] The NUTS classification (Nomenclature of territorial units for statistics) is a hierarchical system for dividing up the economic territory of the EU (see http://ec.europa.eu/eurostat/web/nuts/overview, last accessed 22 August 2016).

Table 10: Examples of databases to characterise the land use/cover in the RPU

Factor	Database	Temporal resolution	Spatial resolution (expressed in the units used in the databases)	Type of data
Land cover	CORINE Land Cover[(a)] (CLC, 2006) (CLC, 2012 pending validation) http://land.copernicus.eu/pan-european/corine-land-cover	2006 ± 1 year (2012 version under validation)	25 ha (Minimum mapping unit) 100 m (width) (Geometric accuracy of satellite data ≤ 25 m)	Satellite data (SPOT-4/5 and IRS P6 LISS III dual date)
Land cover	EUNIS, 2007	2007 year (Revised descriptions 2012)	1 × 1 km	GIS data (According to Habitats Directive 92/43/EEC)
Land cover/use	LUCAS Survey, 2012 http://ec.europa.eu/eurostat/web/lucas/overview	2009–2012 year (Updates every 3 years, 2015 version pending validation)	2 km grid (Including around 1 million points all over the EU)	Point data (survey observations) GISCO system Geographic Information System of the Commission [GIS data + European Statistical System (ESS) + EU Commission]
Land use	The Land Use Database of the Netherlands (LGN7), 2013. http://www.wageningenur.nl/en/Expertise-Services/Research-Institutes/alterra/Facilities-Products/Land-use-database-of-the-Netherlands.htm	2012 (Updates every 3–5 years)	25 × 25 m grid	Aerial photos + satellite images (from the National Satellite Data Portal, NSD) In addition: Land Parcel Information System, 2012 + Kadaster's topographic base date, 2012 + Land Parcel Information System, 2012 + the Netherlands digital land use map, 2008 + Basic Nature Map, 2012
Land cover	Morton et al., 2011 http://digimap.edina.ac.uk/webhelp/environment/data_information/lcm2007_final_report.pdf	1990–2000–2007	25 × 25 m grid	Satellite data (by Landsat 5 Thematic Mapper)

(a): Details on CORINE Land Cover (CLC, 2006) can be consulted in Table C.28.

3.3.2. Environmental drivers (TOR2)



H-HH, factors with a High relevance to bee health, High technical feasibility and High priority; **H-HM**, factors with a High relevance to bee health, High technical feasibility and Medium priority; **L**, factors with Low relevance to bee health. The score H-HH is highlighted in green as the factors with this score are taken forward in TOR3, whereas the other factors not.

Figure 11: Mind map of environmental drivers – identified factors and corresponding scores

The environmental drivers are abiotic factors that have been grouped in three categories: soil, weather and climate (see Figure 11). Collecting data on environmental drivers in field surveys is relevant given their influence on behaviour, demography and in-hive products. Further details are available in Appendix B, Table B.8, where a comprehensive list of the environmental drivers is presented together with their respective variables. The paragraph below briefly describes the 'environmental driver' indicators, in particular those with high scores.

3.3.2.1. Relevance of the environmental drivers to the bee health status of a colony

Weather is defined in short term variations (hours–days–month) of the abiotic factors in RPU (Section 3.3.1) having an effect on the health of a honeybee colony. The activity of honeybees highly depends on the outside temperature (Hatjina et al., 2014a); for instance, a cold temperature is commonly associated with increased stress on bees because bees will not venture out of the hive if temperatures are below 8–10°C, reducing their food intake (British Columbia Ministry of Agriculture, 2012). The reproduction of parasites and pathogens harmful to honeybees (e.g. *Nosema* spp.) depends on the honeybees' thermoregulation capacity and activity (Southwick and Moritz, 1987) and the foraging activity of the bees (Kaur and Sihag, 1994) is influenced by the relative humidity of the RPU. Several studies have provided scientific evidence that total precipitation (i.e. rain and snow) directly affects foraging activity (Blaschon et al., 1999; Schmickl and Crailsheim, 2002; Crailsheim et al., 1999; Van der Zee et al., 2015). For instance, Blaschon et al. (1999) measured the amounts of food stores and brood during alternating periods of good weather (three periods of 6 days each) and bad weather (three periods of 5 days each), which was simulated by intense artificial rain. The amount of stored pollen typically increased during periods of good weather and decreased during bad weather. Solar radiation has been shown to affect the honeybee defensive and foraging behaviour, together with their thermoregulation (Southwick and Moritz, 1987). Because of limited scientific evidence available linking factors, such as wind and atmospheric pressure with bee health, these factors were scored low.

Weather factors (i.e. temperature, relative humidity, precipitations, solar radiation) have a direct effect on the behaviour and physiology of the colony, and thus have an indirect effect on the health status of the colony. The relation between bee health and varying weather conditions has been studied by many authors (Blaschon et al., 1999; Schmickl and Crailsheim, 2002; Crailsheim et al., 1999; Van der Zee et al., 2015).

Climate is defined in medium- and long-term variations (season, year, multiannual) of abiotic factors in the RPU. The factors relevant to bee health that have been reported are the climate type (Köppen climate classification system; Kottek et al., 2006) of the colony location, the thermal sums (degree days) that influence the population dynamics of an insect population, and average temperature and average precipitation (rain and snow) patterns (Blaschon et al., 1999; Crailsheim et al., 1999; Hatjina et al., 2014a). Snow cover affects the foraging activity of honeybees (Moeller, 1977), although foraging does in general not take place at temperatures below 10°C.

The type of soil and its water and nutrient content are abiotic factors for which the link with bee health is considered low.

3.3.2.2. Technical feasibility and priority to include factors on environmental drivers in field surveys

The methods available for measuring single meteorological factors (i.e. temperature, precipitation, solar radiation, etc.) are standardised and described in the Guide to Meteorological Instruments and Methods of Observation from the World Meteorological Organization (WMO, 2008). Table 11 shows two examples of existing databases containing data with different time and spatial resolutions.

3.3.2.3. Methods and tools to measure factors related to environmental drivers (TOR3)

Standardised and harmonised methods exist for the measurement of outside temperature and relative humidity. Further details are presented in Appendix C, Table C.29. The time to take into account has to be defined based on the objective of the survey.

Weather data, mainly collected through weather stations, are available for most EU countries and the low cost of data collection, processing and data transfer from temperature measurement systems facilitates many applications of temperature measurements in beekeeping (Zacepins and Karasha, 2013). Some databases including the meteorological data identified as part of environmental drivers affecting bee health are illustrated in Table 11.

Table 11: Examples of databases available at the EU level for selected factors on environmental drivers. The factors highlighted in green have a H-HH score (see Figure 11)

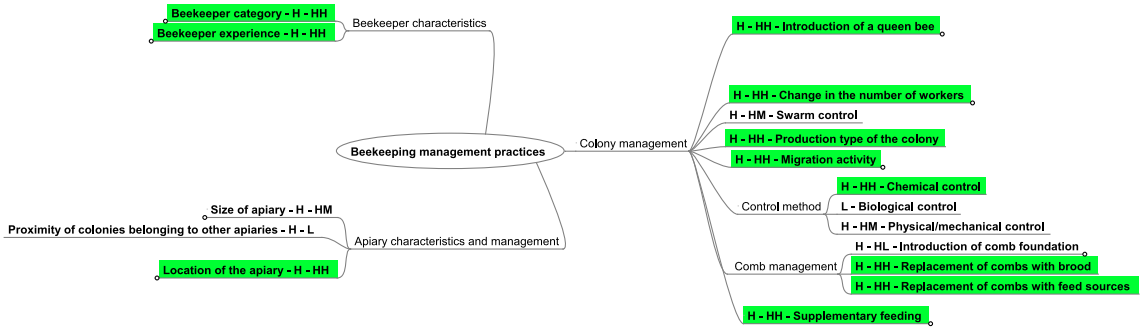
Factor	[Reporting unit]	Database	Temporal resolution (expressed in the units used in the databases)	Spatial resolution
Max, min and mean air temperature	[°C]	MARS-AGRI4CAST (JRC)	Daily	25 × 25 km
Relative air humidity	[%]		Daily (every 3 h)	
Wind speed at 10 m	[m/s]		Daily mean	
Vapour pressure	[hPa]		Daily mean	
Sum of precipitation	[mm/day]		Daily	
Total global radiation	[kJ/m^2/day]		Daily	
Snow cover	[km^2]		Daily	
Climate type	Thermal sums (degree days) Average precipitation Average temperature	Köppen–Geiger climate classification	Long term (months to years)	0.5°
Snow cover	[km^2]	EUMETSAT – Satellite Application Facility on Climate Monitoring – (CM SAF)	Long term (daily to monthly)	4 × 4 km
Precipitation	[mm/days]			

Details are given in Table C.30.

3.3.3. Beekeeping management practices

This section provides a short description of the 'beekeeping management practices' (BMP) that can directly and/or indirectly influence the health of a colony. Further details are available in Appendix B, Table B.8.

This section on BMP describes the human interventions implemented by the beekeeper to ensure proper colony management in order to maintain a healthy colony and, if intended, achieve the production objectives (e.g. including colony productivity in terms of population and outputs). The beekeeper is defined as the person managing the colony throughout the year. He/she provides information and data required in a field survey and should receive a targeted training for data collection and reporting.



H-HH, factors with a High relevance to bee health, High technical feasibility and High priority; **H-HM**, factors with a High relevance to bee health, High technical feasibility and Medium priority; **H-HL**, factors with a High relevance to bee health, High technical feasibility and Low priority; **H-L**, factors with High relevance to bee health and Low technical feasibility; **L**, factors with Low relevance to bee health. The score H-HH is highlighted in green as the factors with this score are taken forward in TOR3, whereas the other factors not.

Figure 12: Mind map beekeeping management practices – identified factors and corresponding scores

As shown in the mind map below (Figure 12), the BMP is an external driver that depends on beekeeper characteristics (i.e. beekeeper category and experience) and specific beekeeping operations (i.e. colony management, apiary characteristic and management).

3.3.3.1. Relevance of the beekeeping management practices to the bee health status of a colony

BMP act directly on the honeybee colony and hence should be taken into account when assessing its health status (Figure 12).

The number of colonies managed by the beekeeper and their economic viability are the variables that the Member States commonly use to define beekeeper category across the EU. As shown in Van der Zee et al. (2012), in most of the countries under scrutiny, hobbyist beekeepers (managing 1–50 colonies) reported higher losses than practitioners with intermediate beekeeping operations (51–500 colonies) (Van der Zee et al., 2012). Deloitte (2013) reports that colony losses lead to economic losses and, because of their unpredictable nature, this source of uncertainty could limit the recruitment of a new generation of beekeepers. Long-term decline in colony numbers could be driven by socioeconomic and political pressure on honey production (Smith et al., 2013). Similarly, this aspect could be influenced by specific socioeconomic factors such as the presence or absence of state subsidies (EASAC, 2015). Thus, the number of beekeepers and their economic status could be both a cause and an effect of an increase/decline in the number of honeybee colonies (Potts et al., 2010; Deloitte, 2013; Smith et al., 2013).

The beekeeper experience is linked to personal beekeeping skills gained through practice and training (e.g. years of experience, number of colonies managed, qualification obtained). This factor influences the ability to understand and cope with the health status of the colonies, as well as the BMP used in specific scenarios. The ability of the beekeeper to manage the colonies influences honeybee health (EPILOBEE 2012–2014, see Jacques et al., 2016) and is therefore scored as highly relevant. It should be taken into account in particular when the selection of apiaries involved in a field study is not done randomly.

Colony management includes the practices used to manage a single colony: introduction of a queen, modification of the number of workers, comb management, swarm control, application of control methods, provision of supplementary feeding, migration activity and production type chosen for the colony (see Figure 12).

Control methods are used by the beekeepers to control infectious agents/pests/predators, however, might elicit negative side-effects on honeybees. Chemical control practices are those related to the use of active ingredients (i.e. veterinary products), including organic acids and essential oils to control *V. destructor*. Active ingredients used in beekeeping could contaminate bee matrices and impair bee health (Mullin et al., 2010; Rosenkranz et al., 2010). Similarly, physical/mechanical control methods are typically used by beekeepers to reduce the impacts of infectious agents/pests/predators on the colony. However, these control methods do not require the use of an active ingredient. Physical control methods could have adverse side-effects on honeybee health if the good management practices are not followed; for instance, the reuse of inefficiently sterilised contaminated non-living material (i.e. hives containing AFB spores) may lead to further contamination later in time (Neumann and Hoffmann, 2008). The use of biological control methods is not common among the EU beekeepers and therefore considered of low relevance for honeybee health. For instance, the use of entomopathogenic fungi and nematodes has been reported to control SHB (i.e. *Aethina tumida*; JD Ellis et al., 2004*;* Richards et al., 2005; Muerrle et al., 2006*;* Leemon and McMahon, 2009*;* Leemon, 2012).

Comb management, especially when it involves the replacement of combs with feed and/or brood, and supplementary feeding directly influence the colony's demography and nutritional status (Barker, 1977b; Mattila and Otis, 2006; DeGrandi-Hoffman et al., 2010; Di Pasquale et al., 2013). The introduction of brood into a colony typically increases the risk of spreading diseases because an infectious agent and/or a pest could be present in the introduced comb and may subsequently spread within the new colony. Furthermore, the feed and the wax introduced could be contaminated by pesticides. Supplementary feeding is provided by beekeepers to improve the nutritional status of colonies; however, it may lead to negative consequences on colony health, depending on colony demography, ingredient's quality and time/duration of administration (Schmidt et al., 1995; Mullin et al., 2010; Pettis et al., 2012).

Changes in the number of workers and swarm control influence colony demography (e.g. abundance of brood and/or adult bees) and could, therefore, influence the infectious agent/pest/predator population (Robinson and Huang, 1998).

The introduction of a queen bee by a beekeeper is a common practice, particularly for commercial beekeepers, and is of crucial importance both for health and trade purposes. Its main purpose is to increase fecundity: young queens produce more brood (Akyol et al., 2009). The genetic origin of the queen can influence the longevity of the workers. It has been reported that colony survival probability increases when queens of local origin are used (Büchler et al., 2014).

Migration activity is a common practice, especially among professional beekeepers. Modification of the colony location exposes the colony to different environmental conditions (e.g. weather, climate), RPUs (e.g. different habitats) and infectious agents/pests/predators. Typically, beekeepers move their colonies to better foraging areas to increase production and/or provide the bees with better nutrition. However, migration activity might be stressful for a colony.

The management practices that a beekeeper applies to a colony typically depend on its production types, such as honey, pollen, royal jelly, propolis, wax, venom, queens, nucleus (queen, workers and brood), bee packages (adult worker bees) and/or pollination services.

The apiary characteristics and management can be described by the location and size of the apiary, and the proximity of colonies belonging to other apiaries (Figure 12). The location of the apiary is essential to define the area surrounding the studied colony (see Section 3.3.1 on RPU). Therefore, it is scored as highly relevant when assessing honeybee health status.

The size of the apiary relates to the number of colonies in the apiary under scrutiny. The proximity of colonies belonging to other apiaries refers to colonies that, originating from other apiaries, are introduced in close proximity (i.e. within the area in which bees from two colonies can interact) to the colony under investigation. These two factors can increase the risk of, for instance, *V. destructor* (and associated viruses) infestation and bacterial infections, due to drift and bee robbing behaviour (Lindström et al., 2008; DeGrandi-Hoffman et al., 2016; Seeley and Smith, 2015; Nolan and Delaplane, 2016).

3.3.3.2. Technical feasibility and priority to include factors on beekeeping management practices in field surveys

In relation to field surveys special attention should be given to the different actors involved in the data collection, in particular the beekeeper and the inspector (see Section 2.2.2). They should be informed about the objectives and reasons for performing the field survey, including the frequency of bee colony inspections, the use of preferably standardised sampling and measurement methods, reporting methods and data protection issues. All factors scored H-HH in the mind map (Figure 12) have a high technical feasibility and priority for data collection in field surveys. These data can easily be collected through a questionnaire, as done in previous studies across the EU (EPILOBEE 2012–2014, see Jacques et al., 2016; COLOSS questionnaire, see Van der Zee et al., 2013). These factors are considered essential to measure factors related to beekeeping management practices (via questionnaire, see Appendix C, Table C.30). The critical point is to get accurate and detailed replies on the questions because the quality of the collected data will determine the quality of the analysis.

As stated above, the number of colonies managed by the beekeeper and their economic viability are the variables that the Member States commonly use to define the beekeeper category. This factor has been included in previous surveys (e.g. EPILOBEE 2012–2014) and to describe the apicultural sector in each Member State (e.g. national apicultural monitoring programmes), although different categories are used. Within EPILOBEE 2012–2014, beekeepers were characterised based on the number of colonies and apiaries being managed, and the income generated by the activity of the beekeeper (Chauzat et al., 2013). The Member States define the beekeeper category in either three (e.g. hobby, part-time and professional) or two (professional or non-professional) levels (Chauzat et al., 2013). According to Commission Implementing Regulation (EU) 2015/1368, 150 colonies is the threshold for defining a professional beekeeper in the context of the EU financial aid provided to the apicultural sector. According to Deloitte (2013), the income received by the beekeeper should be taken into account when categorising beekeepers, but recognises a high variability within the EU Member States. Although some national assessments might be available, there is no comprehensive EU-wide study publicly available to evaluate the economic situation of the beekeeping sector (Deloitte, 2013). The Panel acknowledges that there is no standard and common definition of beekeeper income across the EU, and that the relation between the number of managed colonies and income is highly variable across the EU; this should be considered in relation to the economic context of each Member State.

Therefore, the Panel distinguishes two categories of beekeepers, i.e. professional and non-professional, based on the level of income. A professional beekeeper makes a profit from the activity, by commercialising bee products and/or renting colonies for pollination, whereas beekeeping is not a significant source of income for non-professional beekeepers. The variable retained to categorise beekeepers is whether the activity generates a 'significant source of income'. Furthermore, the Panel acknowledges that data on the number of colonies managed by beekeepers across the EU could be used as additional information when assessing beekeeper category.

An assessment of the beekeeper experience is needed, together with information on beekeeper category, to specifically train the different beekeepers involved in surveillance activities in order to ensure harmonisation across the survey.

With regards to the introduction of a queen bee, the date and reason for the introduction are necessary to understand colony health, specifically colony demography. The introduction of a queen bee is a common and essential BMP across the EU. Information on the queen's origin (e.g. geographical and/or genetic, if produced by the beekeeper) would be very valuable to collect.

Knowledge of the number of adult worker bees is essential to assess colony health. Beekeepers, especially professional ones, commonly change the number of workers in their colonies, and this practice has high priority in field surveys.

The replacement of combs with brood and the replacement of combs with feed sources are common practices among the EU beekeepers (especially professional ones) that influence bee health and its assessment in field surveys, through a direct change in the number of adult bees, brood (if present in the managed combs) and feed sources.

Chemical control treatments are common across the EU, i.e. the *Varroa* population is controlled through annual chemical control treatments.

Supplementary feeding is commonly used by the EU beekeepers (especially professional ones) to help colony health during difficult times of the year, or to increase production.

The location of the apiary is determined by the beekeeper. It is an essential factor that provides information on the RPU and migratory activity. Reporting the location of a colony whenever it is moved, will allow its migration activity to be followed. Once the apiary/colony location is known, databases can be consulted to extract relevant information (e.g. weather and climate).

Migration activity may influence colony health, and is a common practice among the EU beekeepers. Information on this factor could be determined by the factor 'Location of the apiary', through modification of colony GPS coordinates in time.

Honeybee colonies could be managed in many different ways to reach a specific production goal. The production type of a colony provides information on this objective, which influences the BMP used by the beekeeper. Therefore, it is necessary to define the type of colony under study. This factor might also be relevant when assessing 'colony outputs' (e.g. hive rental for pollination services).

Regarding the factors scored H-HM and H-L:

- data on swarm control practices can easily be collected, but were scored as medium because of poor knowledge on their interpretation in terms of effects on honeybee colony demography and health;
- the size of the apiary varies depending on its production goals and geographical location in the EU, therefore, its priority is scored as medium;
- physical control methods are highly relevant for honeybee health (e.g. *Varroa* control), and their implementation is highly feasible. However, their priority for data collection in field surveys is medium because of variability in the frequency of their implementation across the EU;
- information gathered on the introduction of empty combs (i.e. comb foundation) can be used only partially to understand direct effects on bee health because the risk of chemical/biological contamination is mainly linked to the content of the combs rather than to their structure. Its priority was therefore scored as low;
- information regarding the factor 'proximity of colonies belonging to other apiaries' could be gathered through a questionnaire, although accurate data regarding this factor is not easily available to individual beekeepers. Therefore, the technical feasibility in field surveys is low.

3.3.3.3. Methods and tools to measure factors related to beekeeping management practices (TOR3)

In Table 12 the essential factors and related variables to be considered when assessing 'beekeeping management practices' are represented. Further details on the variables related to the factors

describing the 'beekeeper characteristics', 'apiary characteristics and management' and 'colony management' are presented in Appendix C, Table C.30. Measurement of these variables is based on data collected through questionnaires. Questionnaires have been used in previous projects, such as the COLOSS survey (e.g. Van der Zee et al., 2013), NBU Husbandry Survey (2014), USDA APHIS/Bee Informed Partnership Project (questionnaire-based surveys, see vanEngelsdorp et al., 2012), EPILOBEE (2012–2014, see Jacques et al., 2016) and Italy's APENET and BeeNet projects (see Porrini et al., 2016) (field-data sampling based surveys).

The BMP questionnaire could be designed as an online survey (see BeeNet, COLOSS) or with paper support and should ideally be available to the beekeeper throughout the beekeeping season in order to systematically capture all the relevant information. As stated in Section 2.2.2, it is recommended to measure many indicators and factors at least three times during a year: after winter (e.g. 1–2 weeks after bees start foraging but before first big nectar flow), during summer (active season) and before winter (when the colony is preparing for winter). However, it is clear that more frequent data collection will enrich the data set and is recommended whenever possible. The exact timing of the measurements has to be defined based on the objectives of the field survey. Frequent data inputs are particularly required when analysing the effect of migration activity on the health status of a colony. Therefore, data collection through a questionnaire could be envisaged three times a year, and in this case the beekeeper could report back the beekeeping management information recorded (e.g. in the beekeeping record book). Special care has to be taken when the questionnaire is designed to prevent unnecessary repetition of questions in repeated questionnaires as it will reduce the number of completed questionnaires. It should also be checked that any question asked leads to an answer that will be analysed, otherwise there is no point including those. Careful consideration of the level (e.g. colony or apiary) considered by the question should be done in relation to the objective of the survey and the feasibility of getting accurate replies by the participating people. More guidance is provided in Section 5.

Data collection for BMP depends on the timing of the activities during the year. Databases can also be used to measure variables (e.g. the location and size of apiaries can be obtained from the Member State registers, if available). If the variable to be quantified does not need a specific measurement and can be derived from other data provided through the survey, this is indicated. More details allowing the assessment of 'beekeeper characteristics' are presented below.

Beekeeper category

According to Regulation (EU) No 1308/2013, national programmes for the apicultural sector should be drawn up every 3 years with a view to improving the general conditions for the production and marketing of apiculture products. Based on these national programmes, triennial overview reports of the apicultural sector in the EU are published providing details on the number of beekeepers across the EU, the number of hives managed and their economic viability status. The Panel distinguishes two categories of beekeepers, i.e. professional and non-professional, based on the level of income; therefore, the essential variable retained to categorise beekeepers is whether the activity generates a 'significant source of income'. This variable should be weighted based on the economical context of the country. Furthermore, the Panel acknowledges that data on the number of colonies managed by the beekeeper across the EU could be considered as additional information for implementing beekeeper category assessment.

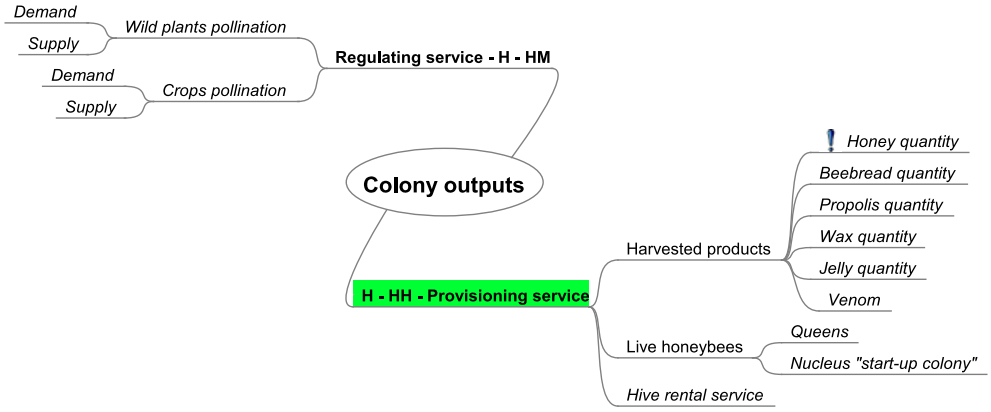
Beekeeper experience

Previous surveys (e.g. EPILOBEE 2012–2014; Deloitte, 2013; COLOSS, 2015) have assessed the experience of beekeepers based on specific variables, although this was not the main purpose of the surveys. For example, within EPILOBEE 2012–2014, beekeepers were characterised based on specific variables (e.g. for how long they have been beekeeper, if they are member of a beekeeper association, if they have a qualification in bee husbandry, if they were trained, and if they used a beekeeping record book). Analysis of these variables is presented in EPILOBEE 2012–2014 (see Jacques et al., 2016). The COLOSS project assessed only the number of apiaries managed by the beekeeper. In Deloitte (2013), the focus is on the years of experience beekeepers have. Considering the difficulty of assessing beekeeper experience, and recognising the wide set of variables listed in Appendix B, Table B.8, the Panel recommends considering three essential variables: 'years of practice', 'number of beekeeping courses attended' and 'bee meetings attended' by the beekeeper. The collection of data related to beekeeper experience combined with the beekeeper category can be used to better correlate beekeepers with the honeybee health status of a colony. In Appendix C, Table C.30, variables on training information, specific trainings and technical abilities are presented.

Table 12: Measurement of selected factors on beekeeping management practices. The variables most suitable for implementation in field surveys across the EU are highlighted in green. All variables can be collected via questionnaire. Details on these methods are given in Tables B.8 and C.31

Factor	Variable [unit]
Beekeeper category	Significant source of income [Y/N]
	Number of hives managed [n]
Beekeeper experience	Technical abilities [see list in Table B.8]
	Training [see list in Table B.8]
Location of the apiary	Location of the colony [X,Y coordinates] at date [dd-mm-year]
	Average distance between the colonies of the same apiary
Introduction of a queen bee	Date [dd-mm-year] and reason of introduction [see list in Table B.8]
	Geographic origin of the queen bee
	Genetic origin of the queen bee (if available)
Change in number of workers	Date [dd-mm-year]
	Quantity of each introduction/removal of bees [g, n; introduction or removal]
	Bee origin [apirary location (i.e. X/Y coordinates, municipality or NUTS3 region]
Production type of the colony	Type of product/activity [honey, pollen, bee packages, royal jelly, queens, nucleus (queen, workers and brood), propolis, wax, venom, hive rental]
Migration activity	Migration effort [number of times the colony location changed during a beekeeping season]
Chemical control	Product/Active ingredient used [selection from list of products used in a given MS]
	Target (e.g. Varroa) [selection from list of infectious agents and pets occurring in a given MS, see Section 3.2.5]
	Application method [solid, liquid, gas, other; see Table B.8]
	Dose/concentration [number (e.g. of strips), mg or mL per colony]
	Duration of the treatment [start dd-mm-year, end dd-mm-year]
Replacement of combs with brood	Action [introduction, removal]
	Date [dd-mm-year]
	Quantity [number of combs or surface covered by feed, brood and adult bees in cm^2]
Replacement of combs with feed sources	Action [introduction, removal]
	Date [dd-mm-year]
	Quantity [number of combs or surface covered by feed and adult bees in cm^2]
Supplementary feeding	Duration [start dd-mm-year, end dd-mm-year if available]
	Type [carbohydrate, protein; see Table B.8]
	Quantity [mg or mL per day per colony]

3.4. Colony outputs (TOR2–3)



H-HH, service with a High relevance to bee health, High technical feasibility and High priority; H-HM, service with a High relevance to bee health, High technical feasibility and Medium priority. !, recommended variable to assess the corresponding service. The score H-HH is highlighted in green as the factors with this score are taken forward in TOR3, whereas the other factors not.

Figure 13: Mind map outputs of the colony: provisioning and regulating services – identified factors and corresponding scores

3.4.1. Relevance of colony outputs to the bee health status of a colony

As indicated in Section 3.1 a managed honeybee colony is considered healthy when the following end points are achieved:

- it has an adequate size, demographic structure and behaviour in relation to the annual life cycle of the colony and the geographical location;
- it has an adequate production of bee products in relation to the annual life cycle of the colony and the geographical location;
- it provides pollination services.

The last two end points are considered outputs provided by the bee colony in terms of ecosystem service provision, as represented in Figure 13.

The amount and quality of the outputs represent the service provided by the bee colony to the ecosystem. Millennium Ecosystem Assessment (2005) defines ecosystem services from an anthropocentric perspective and considers ecosystem provision when humans benefit from the environment. Colony outputs are considered in terms of service provision of the managed honeybees and only the two main ecosystem services directly affected by the managed honeybees are addressed in this opinion.

In this context, bee products correspond to an ecosystem provisioning service, whereas the pollination service provided by the bees corresponds to an ecosystem regulating service. Analysis of the outputs can provide information on overall bee colony performance and an assessment of the health status of a managed honeybee colony should, therefore, include measurement of the outputs in terms of provisioning service – for the harvested products, the hive rental service and the live honeybees – and of regulating service in particular of the pollination services provided by the bees.

The goal of a beekeeping enterprise is to obtain marketable outputs, corresponding to the provisioning service provided by the managed honeybee colony. These outputs can be measured in terms of the harvested products (honey, pollen, bee bread, propolis, wax, jelly and venom), live honeybee production [queens, nucleus (start up colony)] and hive rental service, as indicated in Figure 13.

Pollination is a key regulating ecosystem service and its importance is widely recognised (Klein et al., 2007). Pollination services regulate various benefits such as crop production, non-crop plants and landscape aesthetics. This opinion only addresses the pollination service provided by the honeybees to cultivated and wild plants. Landscape aesthetics and other indirect benefits are not assessed due to the complexity of measuring them. Honeybees visit a large number of plants, and

provide a substantial proportion of pollination services for many of them. The measurement of pollination services to multiple plants within the wider landscape may represent a tangible measure of overall colony health because weak colonies in poor health will often provide little pollination or only provide services to the most beneficial resources (which may include a high yielding crop). However, the foraging preference will dominate the foraging activity and may vary over time and from one apiary to another; this should therefore be taken in account when quantifying the pollination service provision.

The provision of this ecosystem service is a major concern, mainly in the context of declining pollinator populations (Bos et al., 2007). The loss of native habitats for bees also affects agricultural production by degrading the services provided by pollinators (Foley et al., 2005). Liss et al. (2013) reviewed the current literature on the measurement of pollination ecosystem services and highlighted the importance of a clear definition of the ecosystem service by comparing different quantitative measures. A comprehensive definition of pollination services is needed and should take into account the following when evaluating it within the RPU:

- the pollination service provided by the managed honeybees needs to be assessed for both the crops and wild plants that benefit from insect pollination;
- the land cover, plant phenology and plant and flower density, the flower characteristics in terms of their attractiveness to bees and the need for cross-pollination.

The weather conditions should be taken in account, because the weather influences pollination as well as human activity.

3.4.2. Technical feasibility and priority to include colony output indicators relevant to bee health status in field surveys

Measurement of the different variables characterising the provisioning service (Figure 13) are routinely performed by beekeepers and can be easily collected within the context of a field survey. In particular, the quantity of honey harvested from a hive is a good indicator of the service and is highly relevant to all Member States and under most conditions. In contrast to honey, only a small amount of pollen, royal jelly and propolis is present in a colony at a given time (Brodschneider and Crailsheim, 2010). For this reason, it is not recommended that these be measured in large field surveys.

With regards to measurement of the provision of regulating services by the honeybee colony, the time and expertise demanded by the field work involved in most viable methods to evaluate supply and demand make this of low feasibility for most methods. Pollination services are provided by various types of pollinators visiting flowers and, under field conditions, it is not realistic to measure the specific contribution of honeybees.

However, if sufficient data are available on hive locations, modelling approaches are recommended and assessed with a medium priority. Although it is possible to link honeybee colony placement to pollination services using modelling methods, limited information on the impact of colony health on service provision prevents accurate modelling of health impacts on pollination services (but see Becher et al., 2014).

3.4.3. Methods and tools to measure factors related to colony outputs

Provisioning service

As previously mentioned, bee colonies produce different types of products (honey, bee bread, propolis, royal jelly, etc.). Bees store honey for use during both the active season and wintering, and begin to store it in the main body of the hive ('nest' or 'brood chamber'), and when there is not enough space or surplus nectar, in the upper part of the hive (the super). Bees can eat the honey of the super, if needed, but it is primarily harvested by beekeepers. Although it is not common practice, the quantity of honey in the super can be easily estimated by weighing the super before and after harvesting the honey. This is an activity already performed by the majority of the beekeepers to estimate total colony production and, this measurement can be easily included in field surveys. It is also possible to determine the quantity of honey in the super without harvesting it, for instance, by weighing the empty super (including the empty combs) before it is installed in the hive. During subsequent weighing of the super containing honey, this reference weight should be subtracted in order to estimate the quantity of honey. If the hive has several supers, all the calculated masses should be summed, because the goal is to know the total quantity of honey per hive. Placing a queen

excluder device between the brood chambers and the super will allow more accurate results because no brood will be present in the super combs. However, if brood or pollen is present in the super combs, it is recommended that the weight of these resources be estimated and subtracted from the final weight. It is recommended to mark the supers with the number of the hive to correctly relate the quantity of honey harvested with the colony production. There are other methods, more or less accurate, for evaluating the quantity of honey in the main body of the hive and in the super, such as digital image analysis and visual estimation (Imdorf et al., 1987; APENET, 2011; Costa et al., 2012; Delaplane et al., 2013b; Odoux et al., 2014; BeeNet, 2014). More details are provided in Table C.32.

The super contains mainly honey, but in some cases, the queen can also lay eggs in some part of the super (the lower part) and it is then more appropriate to calculate the surfaces. When there is only honey in the super, it might be easier to weight it than to take photos or to use grids. The difference between the weight of a full and empty super gives the amount of honey harvested.

Regulating service

The floral resources are addressed in the RPU in terms of productivity of nectar and pollen (see Section 3.3.1 Resource providing unit mind map). The measurement of floral resources in the landscape should include four steps: (i) measure the number of open flowers in each habitat at a given point, (ii) sample nectar (including water and energy), (iii) sample pollen and (iv) extrapolate upwards.

In order to quantify pollination service provision, the proposed approach is to compare, at the RPU level, the pollination demand of the plants and the pollination effectively supplied to the plants by the bees (Schulp et al., 2014a). Pollination demand is defined as the number of visits required by flowers, weighted by the number of flowers requiring pollination to produce either economically viable crop production or stable populations in the case of wild plants. Pollination supply is defined as the number of visits to flowers within an RPU.

Estimates of pollination demand should be undertaken according to the land cover/use (see Section 3.3.1 on RPU) and the phenological stage of the plants within the RPU at a given point in time. Pollination supply should be measured on pollinated flowers at a given time in the RPU considering crop yield, plant fitness, pollen transfer and pollinator visitation. Schulp et al. (2014b) reviewed the scientific literature and acknowledge the ongoing debate about the importance of honeybees and wild pollinators in the supply of the pollination service.

In measuring pollination demand, two types of methods are distinguished:

- field assessments;
- floral coverage.

In measuring pollination supply, three types of methods are distinguished:

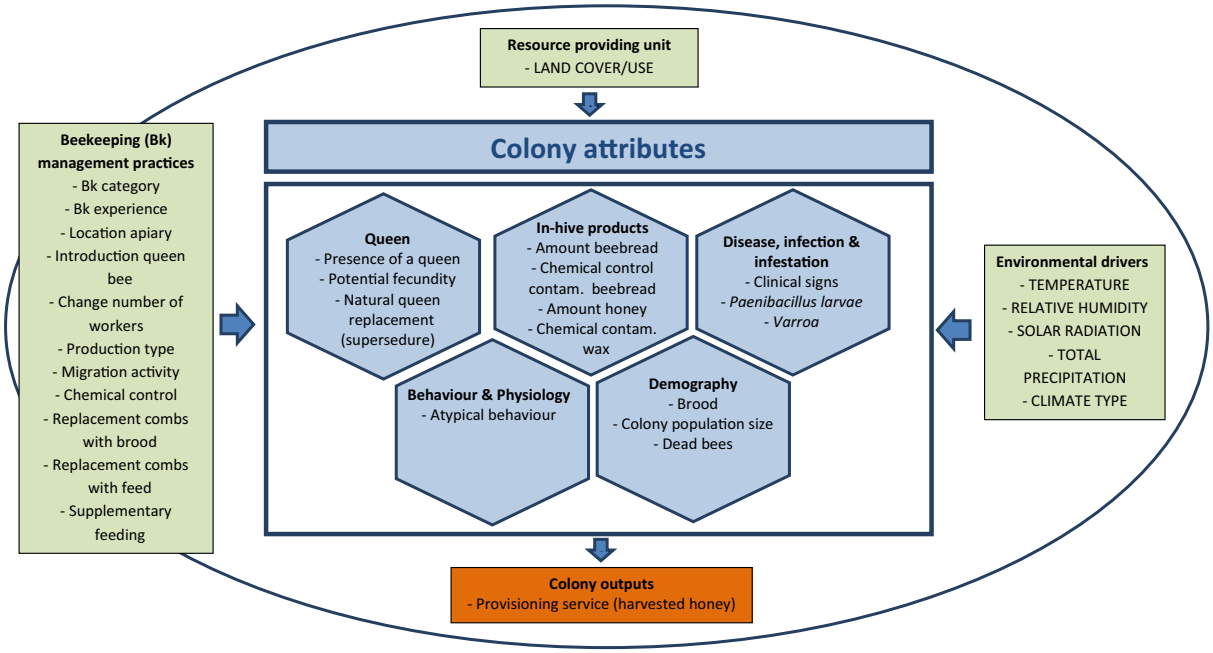
- field-based methods
  - selecting a sample of plants,
  - sampling pollinator visitors to determine the proportion of visits by the focal taxa,
  - conducting pollination assessments to determine deficits;
- in-hive pollen collection;
- modelling methods.

Appendix C (Section 3.2) provides details on these measuring methods and includes their protocols, strengths and weaknesses, and existing data sets.

4. Field data collection: which indicators and factors to include across the EU

Given the need of surveys that take into account all the main characteristics of bee health (including both biological and chemical stressors) and as a summary of TORs 2–3, Figure 14 provides an overview of the indicators and factors with high relevance, high technical feasibility and high priority to include in field surveys across the EU when assessing the health status of managed honeybee colonies in a holistic manner. These are seen as the minimum set of indicators and factors for which data have to be collected across the Member States in order to improve our understanding of their associations with bee health. Depending on the study objective and/or the location within the EU where a field survey will be carried out, the addition of other indicators and factors might be required,

as described in Sections 3.2–3.4 (e.g. SHB in southern Italy). The list of selected indicators and factors needs to be reviewed when new scientific evidence becomes available. Similarly, the methods of data collection and analysis of samples described in Sections 3.2–3.4 need to be regularly updated in view of future technological innovation.



The factors presented in capital letters can be derived from existing databases. (Bk, beekeeper)

Figure 14: Indicators and factors with high relevance, high technical feasibility and high priority to include in field surveys across the EU when assessing the health status of managed honeybee colonies in a holistic manner

As mentioned in Section 1.3, it is not realistic to design a single approach that could be used throughout the EU, but instead create a common toolbox from which one could use the tools relevant to the specific objective of a particular field survey. The toolbox is designed to assess the colony attributes 'presence and performance of a queen', 'demography', 'in-hive products', 'behaviour and physiology' and 'disease, infection and infestation' by implementing methods suited for harmonised implementation in field surveys across the EU. It can be concluded from TORs 2 and 3 that it is difficult to measure 'behavioural and physiological' indicators in field surveys. Only the presence or absence of an atypical behaviour can be assessed at any given moment. Analysis of physiological indicators is only feasible within a research setting using specialised methods and equipment.

Analysis of the RPU has a high relevance to understand the health status of a honeybee colony and is based on defining the land cover/use at the European level because no method is available to describe the RPU around a particular hive in a manner that could be implemented in all Member States. If beekeepers provide the location of their apiary, high level information on land cover/use can be derived from existing databases and hence used in assessments of bee health (see Table 10, Section 3.3.1). Data on agronomic practices and pesticide concentrations in environmental matrices are highly relevant to assessments of bee health, but their collection is currently not feasible across the Member States. Dedicated field and experimental studies, combined with modelling approaches are suited to improving our knowledge. Information on BMP is already collected in several Member States via questionnaires and data on environmental drivers are mainly available in existing databases. Some efforts are required to improve the accessibility of these databases and/or to increase the spatial resolution, to make them useful for assessing bee health within the RPU of a colony.

Regarding colony outputs, provisioning services can be assessed mainly for harvested honey, whereas technical limitations hamper the assessment of regulating services in field surveys across the EU.

The collection of data on the indicators and factors is described separately in Sections 3.2–3.4. However, it is crucial that data collection should be conducted at the same moment and place for

different indicators and factors as they are linked with each other. For instance, data on the use of chemical control methods in the RPU and within the hive should be linked with data on exposure to bees at different places (in plants, in foragers and in the hive). Here, efforts are also required to make existing data (e.g. EU reporting of plant protection products) available for assessing bee health. The use of existing (validated) data (even if collected for another objective), would reduce the number of indicators and/or factors for which active data collections in field surveys is required.

5. Field data collection: considerations during survey design (TOR4)

An analysis that aims to validly compare honeybee health across space and time necessitates a survey design that allows for such comparisons. This chapter provides a brief overview of the key considerations to take into account by anybody who is planning to design a survey. References to relevant documents are provided but involvement of an experienced survey designer would be required.

To validly compare data, the survey should be based on sampling designs allowing such comparison, and data collection should be standardised as much as possible between regions and in time. In this opinion, there is no recommendation on any particular set of choices. It is limited to noting the key importance of:

- carefully designing and implementing each aspect of the survey;
- ensuring ample resources are dedicated the survey. The required resources might be different if the field survey is done as a project or a surveillance activity (one shot versus continuous system) and training should also be considered crucial to limit variability;
- ensuring in advance of any data collection that the design choices fit the desired analyses. For instance, if regional variation is deemed important, then regions should be sampled in a way that reflects this variation; if countries are to be compared, questionnaire translations must yield identical data types; and if time comparisons are needed, editing procedures should be harmonised over time, to name just a few examples. It might be worth considering the involvement of bee inspectors to ensure an appropriate data standardisation for some of the collected variables (e.g. clinical diagnostic and sample taking for some diseases). All the bee inspectors (or at least their supervisors) from the different geographical areas should receive the same training. In addition, the usual statistical requirements of adequate power and informativeness of the sample also apply.

Some guiding principles for conducting comparative surveys can be found in Jowell et al. (2007). Survey choices specific to honeybee health were also discussed by Van der Zee et al. (2013). Recommendations to improve bee health surveillance across the EU are listed by Hendrikx et al. (2009) and cover defining the objectives, organisation of the survey, laboratory analysis, availability of protocols and definitions, data management, supervision and coordination, training, communication, evaluation and performance indicators. Jacques et al. (2016) describes specific recommendations based on the lessons learnt from the EPILOBEE project. Some additional elements that need to be taken into account are mentioned below with reference to available guidance documents.

Standardised data collection is crucial to reduce biases in observations and to control for random variation which will allow for better explanatory and predictive analysis across the local/regional/country levels and better estimates of variability in bee health. Furthermore, in order to appropriately study changes in bee health, explanatory and predictive analyses require data to be collected at different times of year, over several years and in different geographical regions. This challenges efforts towards data standardisation and the implementation of an adapted information system: a well designed database, an appropriate data transmission system from the field to the database and information feedback. The acceptability of the system relies on the simplicity of the data collection and data transmission systems. The training of beekeepers and other people involved in data collection and transmission is also a key issue of the system. When training on a test method is provided, time should be dedicated also to standardisation of the implementation, even if the method is considered simple (non-sophisticated). For instance, visually estimating the colony size can be trained using digital images as it allows determining the deviation between the visually estimated surface covered by bees and the measured surface. Weighing the bees will further help to determine the colony size as precise as possible (see Section 3.2.2). Training should continue until the trainee is able to estimate visually the colony size with the expected accuracy and precision (which is defined according to the objective of the survey).

The underlying organisation of the survey is crucial to ensure its efficiency. A central coordination body should be dedicated to the survey design and its implementation. If the survey has to be conducted at international level, national and local coordination bodies should be included in the general organisation.

Closely related to the specific objectives of the survey, the construction of a sampling frame is crucial and should take into account clustering, stratification, oversampling of specific subgroups (e.g. 'targeted designs'), sample size, power, etc. If the survey aims at the early detection of health events, sampling can also target vulnerable or at-risk groups, according to the concept of risk-based surveillance (Stärk et al., 2006), possibly identified in a previous analysis, in order to detect early signs of deterioration in managed honeybee health. In this case, dependent variable must be selected. The selection can follow methods similar to the 'case–control' design described in the epidemiological literature. Such selective sampling will also affect the statistical model, and the selection process must be taken into account explicitly in the model in order to yield accurate estimates of the quantities of interest. This may be done using survey-type weights or using specialised models (see chapter 24.4 of Cameron and Trivedi, 2005 for an extensive technical discussion).

The questionnaire design requires specific attention because the questions should be unambiguous, formulated in such a way that they are answered as accurately as possible by beekeepers or any other person collecting data, and in a similar way across regions and time. For standardisation it is recommended that a third persons asks the questions to make sure that the beekeeper has well understood the questions and answers all of them. Several principles must be followed to ensure the quality of a questionnaire. First, questionnaire design should be a group effort, involving survey coordinators, beekeeping specialists, epidemiologists, computer specialists and statisticians. This will guarantee its consistency with the survey objectives.

In terms of style, the questionnaire must be easy to read and simple to complete, and care should be taken over the layout (easily identifiable boxes to be ticked or completed, lines to be filled which are sufficiently large and visible).

In terms of content, the questionnaire must first enable the traceability of all data collected. To achieve this, a certain number of obligatory fields are included, such as a single registration number. Supplementary elements concerning traceability must be included in the questionnaire, such as the name of the beekeeper and the person collecting the data if relevant, the date and site of the visit, etc.

Only questions that comply with the survey objectives should be included. Thus, any question that has no direct link with this objective should be eliminated and data that will not be analysed or interpreted should not be collected. This principle will also prevent the compilation of questionnaires that are too long and therefore less acceptable to beekeepers. The internal logic of the questionnaire must be respected; to ensure this, questions should be grouped by category and follow a logical sequence. Several guidance documents are available (e.g. Toma et al., 1999). The translations should guarantee to collect the same data in different languages. Several procedures exist to ensure the correct translation of fields for data collection. One is 'back translation' which is a translation back to the original language of a document by another team of translators to estimate the consistency of the first translation. Another model is translation, review, adjudication, pretesting and documentation (TRAPD), which is a committee-based approach to avoid subjectivity of a unique translator (see Dorer, 2014 for further details).

Once compiled, the questionnaire should be tested in the field, in a real situation, so that any inconsistencies and problems with completion can be promptly identified. Therefore, a pilot study has to be implemented to collect data in each situation targeted by the survey (for the different languages, for the different types of populations targeted and types of people involved in data collection). This pilot study has to be analysed and interpreted by the group of experts responsible for the study design and survey implementation, leading to the final version of the questionnaire. This test might also allow for data validation and integration in the database.

5.1. Data validation

Once the data have been collected in the field, it is essential that they are validated, i.e. that checks are made on their accuracy, so that they can be entered in the database without creating any bias and thus leading to subsequent errors in interpretation.

Validation should be implemented at two levels. The first level should be performed as close as possible to the field, to facilitate corrections and shorten any delays before source data can be

checked. In the case of an international survey, it may be useful to have coordination levels at the national, and possibly local, level for data validation. Thus, in the event of erroneous, absent or illogical entries, it remains possible to ask for supplementary information or confirmation from the beekeeper or the person in charge of data collection. This level of validation consists in looking for missing data, particularly data that ensure the traceability of the questionnaire (form registration number, date, name of farmer); answers should also be checked that appear to be ambiguous (the reasons for ambiguity should be identified and rectified) or are illegible (notably regarding names and figures, as this may have a detrimental effect on results).

A second level of validation must be ensured at the central level of the survey coordination, where supplementary technical resources should be implemented to evaluate the accuracy of the data (standard deviation of data collected, repetitions, test results, etc.).

5.2. Data management and analysis system

The aforementioned stages provide all the elements necessary to develop specifications for the database that will enable data entry and management. The specifications include all fields and tables necessary for the database, together with classification of these data and the links between them. They also include search functions and data-processing functions.

One way of controlling the relevance of the data collected is to define the type of data processing in advance. This will ensure that the data collected are of sufficient quantity and quality to attain the objectives originally fixed for the survey. It will also be possible to detect data of no value, so that data collection and management economies can be made. At the same time, it is necessary to choose the frequency of processing and the methods used to present the results. This will facilitate a very precise definition of the functionalities necessary for the database and the decision tools for data analysis.

6. Field data collection: options for data analysis (TOR4)

6.1. Background

Analysis of bee health should simultaneously cover several indicators and attributes because they are linked directly or indirectly to each other (e.g. demography and behaviour with honey production; disease infestation with bee mortality). Stratification of data (e.g. for genetic origin of a subspecies, production type of the colony) should be considered when analysing data on bee health.

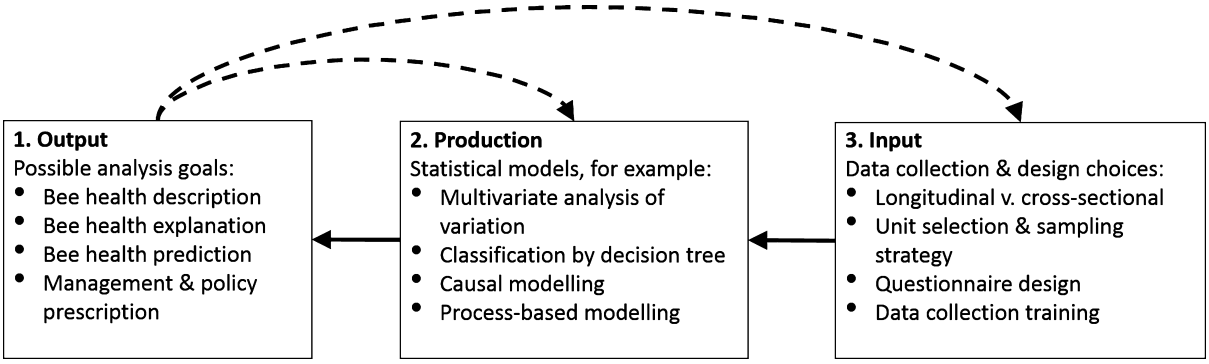
A conclusion in this opinion is that the colony attributes 'presence of a queen', 'demography', 'in-hive products' and 'disease, infestation and infection' can be measured in a harmonised manner under field conditions across the EU. A harmonised measure of the colony output 'pollination service' is still in development, where field measurements can be complemented by model-based assessments (including the uncertainty associated with model errors). A classification of colony health status can be based on direct measurement of these colony attributes using relevant indicators or on combinations of various measured and model-based assessed colony attributes, for example from image analysis of photographs from colonies or land use information from the RPU.

The recommendation for harmonised monitoring of health status is to carry out a minimum of three colony visits per year, with fine tuning depending on the objectives of the survey. From a statistical point of view, this means that a colony can be inspected several times, which allows for longitudinal analysis at the colony level. Some of these measures are made by the beekeeper, accompanied or otherwise by a bee inspector. Because honeybee colonies are managed, the statistical analysis should take into account management carried out by the beekeepers between inspections. Estimates of the importance of early signs of deterioration in order to detect future anomalies in health states may change if BMP are omitted from the analysis.

The purpose of this section is to give a short overview of sensible approaches to the bee health analysis. Because there are many such approaches (e.g. see vanEngelsdorp et al., 2013), it is chosen here to describe and discuss reasonable choices during the steps to set up an analysis of bee health. Where appropriate, examples are given of existing bee health analysis efforts that have adopted one set of such choices.

Here, the analysis of bee health is divided into steps to identify output, production and input (Figure 15). An analysis process flows from right to left, starting with the input (data), through the production (modelling) to the output (goals). An analysis of bee health starts with a definition of the goals and purpose of the analysis, which then, informed by the type of data it is possible to collect, helps to select the modelling approach and data collection effort needed to achieve those goals.

The input phase has been covered in Section 5. The remainder of this section discusses the output and production phases of the analysis. Following TOR4, the focus is strongly on the centre part: the production of statistics and possible modelling approaches.



The type of output determines how to set up the production and what input is required (dashed lines). Outputs (solid lines) are then produced from inputs.

Figure 15: The process of setting up an analysis of bee health starts with identifying analysis goals depending on the type of output that is asked for

6.2. Analysis output: goals of a bee health analysis

What analysis to perform depends on the possible use of the HEALTHY-B toolbox. Goals for an analysis can be descriptive, explanatory, predictive and prescriptive (Figure 15). Here, these goals are briefly elaborated, adapted to the analysis of bee health with examples of existing studies that fit each category.

6.2.1. Descriptive

A descriptive question could be: What is the current bee health status?

The characteristics of a healthy managed honeybee colony are described in TOR1 (Section 3.1.1). In this section of the opinion, the focus is on which methods could be used for a descriptive analysis of bee health, indicating whether bees are doing well, intermediate or badly. The definition of health status may differ between studies and can make a large difference to the study outcome. For example, EPILOBEE compared winter mortality rates in one year with those in a previous year and found few differences or a slight improvement. However, the results may be different when presented as absolute levels or when compared over short or long periods (e.g. 1 or 20 years). Note that the description of bee health is a condition of a colony that is alive, which has an impact on mortalities rates at apiary or local level. The HEALTHY-B toolbox suggests which indicators and factors to measure in order to characterise health in a holistic manner and facilitate detection of health problems before the colony is in a condition that future development and/or survival of the colony will be significantly hampered.

6.2.2. Explanatory (sometimes called 'diagnostic')

An explanatory question could be: What causes changes in bee health?

Explanatory studies seek a mechanistic (causal) explanation for changes in bee health and are typical of scientific publications. Explanatory analyses improve our understanding of the system, which is important to identify management options for safeguarding bee health. Scofield and Mattila (2015) and Clermont et al. (2015) are examples of explanatory studies on foraging and colony losses and weakening, respectively. Explanatory studies include ranges from evaluating associations (correlations) to assessing causations between the indicators for bee health (including health scores) and the factors BMP, environmental drivers and the RPU. EFSA's own efforts in MUST-B seek to explain changes in bee health using analysis informed by a mechanistic understanding of the system.

6.2.3. Predictive

A predictive question could be: What will bee health be like in 10 years?

A predictive analysis simply seeks the best prediction of future bee health, without necessarily asserting causality. Predictive models that associate predictors with bee mortality, such as those coming out of the COLOSS group (Van der Zee et al., 2015), can be seen as part of such efforts. A predictor is any type of variable used to predict health; it may, for instance, be an environmental driver or BMP. In some circumstances, an attribute or indicator described in TORs 2 and 3 can be a predictor of another attribute or indicator. For example, high *Varroa* infestations can explain or predict some queen losses or winter mortalities. Analysis of colony mortality, such as that done in the EPILOBEE project, estimates the association between some drivers and management practices to attributes and mortality rates (Jacques et al., 2016). Henry et al. (2012) assessed the impact of pesticide on colony growth based on field measurements on individual bee mortality. Although these analyses are essentially predictive, to our knowledge, there are currently no studies that explicitly produce predictions of future mortality rates at a colony level.

Examples of predictive analyses of honeybee health that consider impacts and dependencies between more refined colony attributes are even fewer. A predictive model can be less accurate because it does not consider causality between attributes (see Appendix H, approach 3).

Another type of predictive analysis is the mapping of visitation rates by foraging bees, which rely on spatially explicit modelling of foraging behaviour in combination with land use dependent floral information (Schulp et al., 2014b; Koh et al., 2016). Predictions of future visitation rates can be produced by running such a model on future land use scenarios, where flower density is associated with land use. However, the validity and reliability of these models are difficult to verify due to a lack of land use information distinguishing different types of floral resources and with a resolution at a smaller scale than the foraging distances of bees (see Section 3.3.1). As explained in Section 3.2.4, assessing foraging/visitation rate may be done under controlled field conditions ('experimental settings') but it requires a significant amount of time.

One difference between predictive and explanatory analyses is that predictive analysis aims to predict the health state of as yet unobserved colonies. A predictive model should undergo adequate validation of its predictive ability and come with a clear description of its limitations and model uncertainty, before being put into use in decision-making.

6.2.4. Prescriptive

A prescriptive question could be: What management should be enacted to improve bee health in the future?

The prescriptive goal is about providing decision support to beekeepers, regulators or policy makers. Given that a mechanistic understanding of what causes good or bad bee health has been reached, managers can take actions to improve the honeybee health status, possibly in combination with other decision objectives. Some reports have taken this step, such as the OPERA 'Bee health in Europe – Facts and figures' report, which progresses from description (Chapter 2), through explanation (Chapters 3–5) to policy implications).

The mind maps in TOR3 provide a starting point for a mechanistic understanding. These mind maps express possible causal relations among drivers, management, attributes and outputs, but do not express all possible interactions, such as those between different drivers or interactions between indicators under different colony attributes. The mind maps also do not provide insights into relative importance. Development of the mind maps into networks expressing possible and relevant causal links between variables is a possible starting point when the aim is a prescriptive analysis. Such links are currently set up in the MUST–B project. Links can be tested against data, by comparing a model's explanatory performance with and without a link (or set of links).

Clearly, the four analytic goals are not mutually exclusive and can complement each other. Prescriptive analysis requires predictive models, which in turn are built upon explanatory analyses. An example is the MUST–B project, which seeks to go from explanation to prescription, and the work of the COLOSS honeybee research association[31], which seeks to use description to discover explanations and predictions.

[31] http://www.coloss.org/ (last accessed 7 July 2016).

6.3. Analysis production: approaches to modelling bee health

The rationale behind TORs 1–3 is that honeybee health cannot be measured by a single system component. There are no a priori key variables representing univocally the health status, and because health status is influenced by many variables and their interactions, multiple indicators should be considered jointly in an analysis of bee health. The choice of indicators and the relative weights assigned to each of them are crucial for the outcome of the bee health status assessment. Both elements involve a certain degree of subjectivity since they cannot be derived from available data because there is no gold standard to refer to. Therefore, expert decisions will be required to determine rules on how to define and determine health status. Weighting the data will be required, similar to other methods that aim to integrate different indicators into a smaller subset or into one single index. This involves the assignment of a level of importance to an indicator (preferably based on data), relative to the other indicators in the same subset or index. Finally, for practical management purposes, it is essential that threshold levels are determined to trigger action from the beekeeper. The values underpinning the thresholds cannot be determined mathematically but should be the result of consultation between different experts to balance for instance what is feasible and desirable in a given area. When more data would become available in the future, decisions based on expert opinion might be gradually replaced by data-based decisions. Different techniques could be used to integrate indicators into one index (e.g. Spoolder et al., 2003). Two ways in which a HSI could be established are proposed in Appendix H: multivariate analysis and decision tree analysis.

This opinion suggests targeting bee health at the colony level. This means that the analyses covered here should be able to describe, explain or predict future health at the colony level. Health at colony level can be the average health status of a hypothetical colony given a set of factors and indicators, or the health of a specific colony of a particular beekeeper. Health status based on summary statistics of apiaries or regions may be too coarse for an analysis to capture the mechanisms that allow us to estimate the importance of drivers and management factors. Having the colony as the unit of interest implies that observations of colony attributes are colony specific, which explains why some colonies in an apiary could show poor health, whereas others are of good health.

Some indicators are naturally associated with a particular colony, such as the age of the queen and presence of *Varroa*. Others are often regarded at the apiary level. For instance, pollination service can be measured at the colony level via analysis of bee bread/pollen or by taking into account other colonies within the RPU. Model-based assessments could be used although it can be difficult to distinguish the contribution made by different colonies in an apiary.

In the end, regulators and policy makers are interested in health status aggregated to a level higher than the colony, such as apiary, local, regional or country levels. Information on health at colony level can be aggregated into health scores for apiaries up to Member State levels.

The data analysis should also provide means to determine the ranges of spatial and temporal variability in order to forecast under different scenarios and identify any deterioration in health before corrective management actions can be taken. Historical data are limited in the sense that they can come from different data collecting schemes with different associated errors, and can in fact, differ from what is collected in a survey. Therefore, the best option would be to assess the range of variability based on collected longitudinal data as these become available.

To summarise, the analysis of bee health should preferably:

- base the assessment on several indicators and factors;
- use the honeybee colony as the unit of interest;
- assess bee health at different temporal and spatial scales;
- be able to detect early signs of deterioration in honeybee health.

In Appendix H, four approaches are described that could be used when analysing bee health data:

- approach 1 – quantify bee health as a latent variable from multivariate analysis;
- approach 2 – classify bee health in a colony using a decision tree based on the colony attributes;
- approach 3 – predict bee health by causal modelling;
- approach 4 – predict bee health by process-based modelling.

7. Use of the toolbox for different objectives and by different stakeholder groups

As stated earlier in this scientific opinion, the generated toolbox is part of a larger process to achieve EFSA's objective to evolve towards an integrated, holistic risk assessment approach for bees. Efforts to improve data collection, reporting and analysis across the EU will facilitate risk assessment on bee health by national and European risk assessment bodies. The toolbox is in line with the EFSA

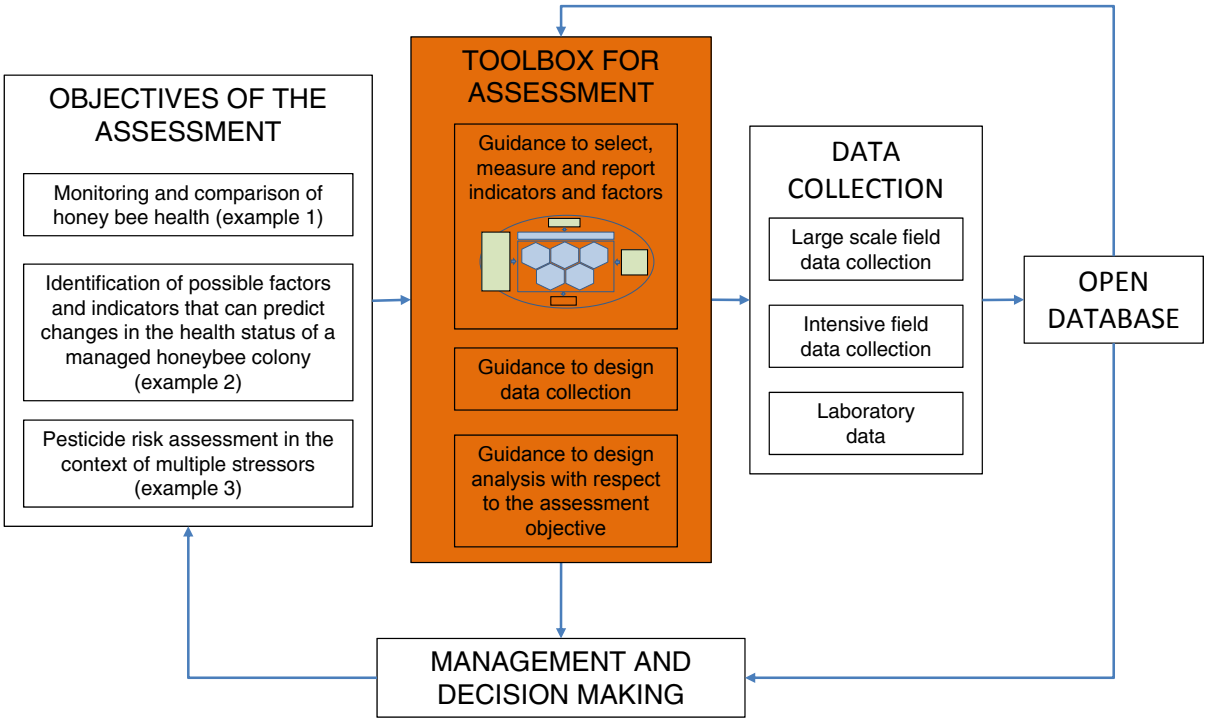


Figure 16: Selecting, measuring and reporting of indicators and factors related to bee health

Strategy 2020 to engage with stakeholders and optimise access to data. The collection of data can be done in the field (via monitoring and surveillance activities (limited number variables in a large area) or via intensive field experiments (more variables and/or more sophisticated methods used in a smaller area) and/or in the laboratory (Figure 16).

Designing of data collections and analysing the obtained data sets are components of the HEALTHY-B toolbox, which can be used in bee health assessment, even if the objectives are different.

The use of the toolbox by different stakeholder groups is described in the sections below using three concrete examples:

1) Monitoring and comparison of honeybee health over time and across geographical space: it is explained how the toolbox could be used to generate a Health Status Index (HSI), which integrates data of several indicators of bee health and factors that determine the health status of the colony.
2) Identification of possible factors and indicators that can predict changes in the health status of a managed honeybee colony: it is explained how the toolbox could help to identify key predictors of a future change in the HSI of a colony
3) Pesticide risk assessment in the context of multiple stressors: it is explained how the toolbox could be used to include also non-pesticide stressors into future pesticide risk assessments

7.1. Example 1 – Monitoring and comparison of honeybee health over time and across geographical space

7.1.1. Background and objective

Many beekeepers monitor the health status of their colonies by assessing (sets of) individual indicators to evaluate whether any intervention is required to obtain maximal outputs from the colony (which could be, for instance, bee products or pollination). As explained in Section 3.1.1, assessing the health status of a honeybee colony requires the integration of several indicators. Many beekeepers probably developed their own approach to select which indicators they take into account and how they integrate these reach an overall health evaluation, making use of their own knowledge and experiences and gathered information (e.g. provided by beekeeping associations and laboratories) (Chauzat et al., 2013; Jacques et al., 2016). The variety of approaches and different management strategies over time and across geographical space hampers the comparison of bee health data within and between apiaries. For example, the concept of a dead colony may vary from the absence of any living bee in the hive to the remaining of various quantities of bees, with or without a queen. But even if commonly agreed definitions and data collection processes are standardised at international level, each indicator or factor will only capture one aspect of bee health. Colony mortality for example, acknowledged to be a major indicator, does not integrate production or the variety of population dynamic status that can be observed. Therefore, the objective of the example below is to explain how the HEALTHY-B toolbox could be used for the design of a robust and harmonised approach aiming at describing a colony's health status and comparing of bee health statuses over space and time. In this opinion, a possible approach is described to generate an health status index (HSI).

7.1.2. What is an HSI for managed honeybee?

Several indicators can be measured from a colony (see Section 4) and the resulting data should be merged in the analysis to define the health status of that colony. Data on chemical contaminants, infection or infestation are not included here in the HSI because presence of 'low levels' of these indicators do not necessarily lead to poor colony health, but in combination with the impact of factors or external drivers influence the health status of the colony.

The outcome describes whether a colony is alive or dead and to what health category the colony belongs (e.g. very good, good, poor or weak). The HSI is determined at the colony level, but can be aggregated at the level of interest (e.g. several colonies within an apiary or several apiaries at local, regional or national level). For instance, a beekeeper might be interested to track the health status evolution of one or more colonies within an apiary, whereas a risk manager might be interested in knowing at a more macroscopic scale which areas have a poorer bee health status, in order to set up specific monitoring and/or mitigation actions.

7.1.3. How does the HEALTHY-B toolbox help to generate an HSI?

The HEALTHY-B toolbox describes two analytical approaches that could be used to derive an HSI for a managed honeybee colony. There is no recommendation given on which approach to use, because this is dependent on the intended use of the index and on the amount and type of available data.

- The first approach is a classification of health status into HSI categories using a decision tree. Appendix H gives a demonstration of how the health status of a colony could be determined by integrating data on the attributes 'presence and performance of the queen', 'colony demography', 'behaviour and physiology', 'disease, infection and infestation', 'in-hive products' and 'colony outputs', such as pollination services. Besides the differentiation of living colonies into ordinal health categories, there is also a need to define when a colony is dead or censored. The latter category is relevant because there is considerable censoring of colonies in field surveys, whenever a queen is replaced by the worker bees or the beekeeper, or when the queen has left with a swarm.
- The second approach aims to generate an HSI using latent variables emerging from multivariate analysis (Appendix H, approach 1). In addition, it is explained in the beginning of Section 6.3 how aggregation of the HSI from the colony to a higher level (e.g. apiary or country) could be achieved.

It is suggested that the HEALTHY-B toolbox be used to select a broad panel of indicators to ensure that bee health is assessed in a holistic manner. Of the indicators identified as most relevant, technically feasible and high priority for inclusion in field surveys across the EU (H-HH indicators, see overview figure in Section 4), the HSI could be constructed from those describing the characteristics of a healthy colony, as described in TOR1 (Table 13; see Appendix H, approaches 1 and 2).

Table 13: Indicators selected from the HEALTHY-B toolbox that could be included in a HSI

Characteristic of a healthy managed honeybee colony	*Attribute/colony output*: identified H-HH indicators and (sign of correlation with good health)
Adequate size, demographic structure and behaviour	*Queen presence and performance*: presence of a queen (+), potential fecundity (+), natural queen replacement (–/+) *Demography*: brood (+), colony population size (+/–), dead bees (–) *Behaviour and physiology*: atypical behaviour (–)
Absence of clinical signs	Presence of a disease (–)
Production of bee products	*In-hive products*: amount of bee bread (+), amount of honey (non-harvested) (+) *Colony outputs*: provisioning service (harvested honey) (+)
Pollination services	Pollination service providers (+)

H-HH: indicators with high relevance to bee health, high technical feasibility and high priority for inclusion in field survey across Europe; –: negative correlation with bee health; +: positive correlation with bee health.

7.1.4. How could the HSI be used?

Once a harmonised HSI has been established, monitoring and comparison of honeybee health in time and space could be implemented across the EU and would be useful for different stakeholder groups. The generation of an HSI would make it possible for beekeepers to monitor their own colonies with the purpose of identifying early signs of possible health problems, so that a beekeeper could intervene and take action to improve colony health and outputs. It would also help to identify regional differences in terms of interactions between stressors as worst combinations for bee health. The HSI could also facilitate the early detection of possible health problems at a local level, which is complementary and possibly much more relevant for early warning and early reaction than monitoring average colony mortality rates because the HSI measures the health of a living colony. Furthermore, the HSI would create, in a harmonised way, the possibility of identifying geographical areas where health is poor and any additional stressors could be more hazardous to bee health than in other regions. The HSI could also be used to monitor how bee health varies over time and space (at a colony up to country level) to understand ranges of variability in health states. This could help to answer questions from risk managers and scientists, such as: (i) is there a real decrease in the number and/or rate of weak/dead colonies over time, (ii) has the health status recovered or improved over time, or (iii) what variation in bee health can you expect over a 5-year period at a given location? The introduction of strata (e.g. beekeeper category, climatic zones, type of agronomic farming practices in the RPU) could allow comparison of the HSI for different categories (e.g. professional versus hobby beekeepers, Mediterranean versus maritime climate zones).

Some possible limitations need to be taken into account when developing a descriptive HSI, in particular regarding the selection of the indicators to be taken into account into the analysis and the interpretation of the results (as is the case for the Welfare Quality project[32]). To avoid confusion and increase transparency, it is recommended to keep the HSI simple and to communicate which individual indicators can be observed directly (and thereby are easy to understand) together with the HSI. It is possible to use maps to communicate the relative proportion of HSI categories aggregated at regional levels (e.g. NUT3 levels) showing trends over time and regions where bee health is at risk or not.

Once a harmonised system to monitor and compare honeybee health in time and space is available, it would facilitate the analysis of which indicators and factors describe or predict the health status of a honeybee colony. Large harmonised data sets might allow testing for single indicators capable of identifying poor colony health on their own and, as a consequence, summarising a holistic description of bee health using the HSI. For example, honey production and/or colony size (in the absence of swarming) and demography could be considered as a proxy for the health status of the colony,

[32] http://www.welfarequality.net/everyone(last accessed 7 July 2016).

assuming optimal conditions of external drivers and absence of stressors. Under those conditions, the HSI could be validated by comparing its outcome with the prevailing data on honey production and/or colony size and demography.

The two other examples also explain possible applications when a HSI is generated.

7.2. Example 2 – Identification of key predictors of change in honeybee health

7.2.1. Background and objective

It is clear that exposure of a honeybee colony to *P. larvae* or a toxic pesticide will negatively affect its health status. However, the cumulative effect of many other stressors on bee health is less clear. For example, it is currently not well understood why *Nosema* spp. can lead to colony mortality in southern Europe and not in northern Europe. The identification of external drivers (e.g. weather, resource availability or beekeeping management practices (BMP)) and/or infectious agents that might lead to a subsequent change in health status once the colony is exposed (further referred to as 'predictor') is difficult, at least because there is not yet a harmonised system to monitor and compare honeybee health status over time and space, that simultaneously considers multiple drivers.

The objective of this second example is to explain how the HEALTHY-B toolbox could be used to identify possible factors and indicators that can predict changes in the health status of a managed honeybee colony.

7.2.2. How does the HEALTHY-B toolbox help to identify key health (status) predictors?

The generation of an HSI, as described in Section 7.1, provides a health status response variable for further analysis with the aim of identifying key predictors of managed honeybee health. Considering key predictors allows for gaining a deeper understanding of bee health and more accurate forecasting of bee health. Explanatory and predictive analysis requires data on the colony's exposure to external drivers and infectious agents collected over space and time.

A key predictor may be important only in relation to the state of another factor or indicator, and therefore, when identifying key predictors, it is recommended that multiple predictors, possible causal relationship between them and between predictors and indicators are also considered. The mind maps from TOR2 offer a means to identify possible causal structures behind such an analysis. Possible key predictors of colony health can be found among the relevant, technically feasible and priority factors beekeeping management, environmental drivers and the resource providing unit (RPU) (see Section 4). There may also be key predictors among the relevant, technically feasible and priority indicators, which predict the state of all other indicators and attributes of a honeybee colony.

Causative and/or process-based models can be used to analyse data, as described in Section 6, to identify and further monitor key predictors. The outcome of the analysis can be presented by estimates of the level of key predictors at the local, regional or country levels, either as trends or maps of Europe, in a manner similar to that suggested for the HSI in Section 7.1.

There are already many ongoing initiatives within the EU collecting data on bee health. The use of the toolbox could ensure to collect standardised data when similar variables are to be collected from one initiative to another. Nevertheless, the variety of objectives of these initiatives might lead to the collection of different sets of data, hampering the possibility to calculate and monitor a HSI in all locations. It could be then necessary to harmonise the different initiatives in order to ensure they are meeting the common objective of calculating an HSI for example. Progress could be made if the toolbox is further developed by collaboration between the EURL, the Member State organisations and EFSA.

7.2.3. How could prediction of changes in bee health status be used?

Given a validated predictive model, it is possible to forecast honeybee health based on projections of key predictors for given areas (e.g. regions or Member States). The result can be communicated as coloured maps showing administrative regions (e.g. NUTS3 level) or ecoclimatic regions. If asked for, the analysis should also be designed to provide uncertainty in forecasts in terms of reliability in regional or strata-specific forecasting, and ranges of uncertainty in the final output from an assessment model.

When the aim of the analysis is to support prescriptive management strategies, it is worth identifying predictors that can be influenced by beekeeping management practices or by land use

decisions or farming practices decisions within the RPU. Risk managers, beekeepers and scientists have an interest in being able to identify a set of predictors that affect bee health and hence should both be targeted by management actions to avoid any future deterioration in bee health. Ideally, it would facilitate the detection of possible health problems at an early stage, at both the regional and the Member State level, to inform policy, but also at the apiary and colony level, as a decision support for beekeepers to prevent unplanned colony mortality or enhance colony outputs, such as pollination services or harvested honey production.

The system is supposed to enable the identification of high/low-risk areas by risk managers and scientists. At the bee population scale, it would then facilitate the selection of relevant sites to study which indicators/factors mainly influence the bee health status and help risk managers to identify regions where management actions are required to improve bee health. At the scale of a beekeeper, it is possible to develop the tool should be designed as a decision support system that, ideally, allows beekeepers to insert data from their colonies to assess whether they need to take action, for instance, through an hand-held device or mobile phone application. For example, climatic conditions combined with a specific landuse structure in a given area might predict a decrease in HSI due to food shortage that could be coped by increased feeding practice by beekeepers. In this context of weakened colonies, additional factors might be of increased influence, such as the presence of risk crops due to usual pesticide management for example (rape).

Such a decision support system should, at best, be developed during an interactive process with beekeepers to incorporate their feedback and knowledge, and facilitate communication and future use of the tool. If the system does not enable beekeepers to get something in return when submitting data (such as risk maps or management recommendation), their motivation for data reporting could decrease dramatically over time. Sustainability is a key issue that has to be considered thoroughly when implementing the system. The advantages of such a system would be to seek the active participation of beekeepers in data collection, which would promote volunteer (i.e. beekeeper) involvement in science. Efficiency of such a system has been already proved in the US with the Bee Informed Partnership[33]. By providing data on their operations, beekeepers would have access to indicators expressing the status of bee health in their apiaries and predicting the health evolution of by integrating also other external drivers, giving beekeepers tools to manage their operations. The possibility of linking data collection to decision support is likely to enhance the engagement of beekeepers and decision-makers towards data collection. These developments should be considered during design of the information system mentioned above. The information system would allow an optimisation of data collection/reporting in relation to bee health and making data accessible would serve many applications by different stakeholders. The Bee Health Workbench project[34] (coordinated by DG-CONNECT) intended to show how data provided by various initiatives could be presented together using common templates, providing also some statistical analysis of data provided. The common use of the HEALTHY-B toolbox by the various data providers would reinforce the relevance to present the data on such a workbench. Nevertheless, one should be very cautious ensuring that data coming from various providers are really adapted for comparison using statistical analysis. Furthermore, interpretation of the statistical analysis should be guided in order to avoid misinterpretation. For example, the absence of a statistical link between two variables could be the consequence of a real absence of link or a lack of statistical power of the analysis or biases in the available data. To illustrate this, in the Bee Health Workbench, presence of American foulbrood apparently reduces the mortality risk according to the linear regression presented (http://172.99.69.60/ScatterPlot-Mortality-Pathogens-Foulbrood.php), which is most probably due to the way the statistical analysis is performed, the presence of confounding factors and the quality of the data used more than a biological evidence.

7.3. Example 3 – Pesticide risk assessment on honeybee health in the context of multiple stressors

7.3.1. Background and objective

However, the effect of pesticides on bee health can be influence by, for instance, infectious agents (ANSES, 2015). In order to make the pesticide risk assessment reflect field conditions more closely, a

[33] https://beeinformed.org/, last accessed on 26 August 2016
[34] see http://172.99.69.60; background available at http://172.99.69.60/About.html, last accessed on 26 August 2016

more holistic approach is required (EFSA, 2013) taking into account the practical feasibility of its implementation. A new paradigm for pesticide risk assessment with a holistic view of honeybee colony heath requires a modelling approach designed to assess the risks from pesticide exposure on a single honeybee colony in a complex environment, also considering the contribution of other stressors, such as biological agents, environmental drivers and beekeeping practices (EFSA, 2016b). There is also analysis of the effect of pesticides on bee health within the Member States, although there is a large variety in the approaches used.

The objective of this example is to explain how the HEALTHY-B toolbox might be used in the development of a new paradigm for pesticide risk assessment on honeybee colonies considering multiple stressors.

7.3.2. How does the HEALTHY-B toolbox help to introduce a holistic perspective into pesticide risk assessment?

Based on the toolbox, the MUST-B WG recently published (EFSA, 2016b) a conceptual process-based model to assess risk to honeybee colonies from exposure to pesticides under different scenarios of combined stressors and factors. The model parameters were derived using the mind maps presented in Section 3 of this opinion. The presented scores on the relevance of an indicator or factor in relation to bee health were considered, whereas the technical feasibility scores were less relevant as they were determined for implementation in a field survey. In addition, the HEALTHY-B toolbox provides recommendations on model structure and calibration in order to facilitate model implementation. In particular, process-based models might be useful when considering casual relationships and expressing variability, as described in Appendix H – approach 4. When high values are at stake and evidence inconsistent, it may be necessary to rely on risk assessment models developed by independent experts and evaluated for scientific rigour.

It is clear that data from both field conditions and laboratory experiments are required to inform the parameters of the 'MUST-B' model. The HEALTHY-B toolbox provides recommendations on survey design for field studies and monitoring the collection of data for use in model calibration and validation to ensure applicability of the toolbox in the different ecoclimatic regions of Europe (Section 5). Intensive data collection at a some places in Europe, representative of regions and climatic conditions, would be required to inform the parameters of the 'MUST-B' model. However, an epidemiological study involving many apiaries across the EU would provide complementary information to analyse the relative importance of different stressors; this information could then be incorporated in the model. Finally, the HEALTHY-B toolbox could facilitate the harmonisation of post-market analysis for instance by the generation of a Health Status Index as described in Section 7.1.

7.3.3. How could a holistic pesticide risk assessment be used?

The incorporation of a more holistic approach in pesticide risk assessment would be useful for risk assessors to assess risks under more realistic conditions, and for risk managers, to take into account the impacts of multiple drivers or stressors when formulating appropriate regulations. Pesticide risk assessment should aim to reflect field conditions, where different stressors can act simultaneously (with cumulative or multiplicative effects) on bees. Models offer an opportunity to overcome many of the limitations posed by field experiments and measurements, particularly the difficulty faced when seeking to interpret the complexity of a honeybee colony and its associated stressors. Because larger data samples are collected over a range of field conditions, a harmonised approach would allow for a more efficient use of field and laboratory data. When a reliable and validated assessment model is established, it may also reduce the amount of data required to assess pesticide risks to bee health. However, at least in parallel with generating models, many test methods should be further developed and validated allowing the collection of precise and accurate quantitative data that are required as input into such models.

A holistic assessment seeks both realism and understandability, and must therefore use a model structure that balances simplification and complexity. Developing, validating and implementing such a new approach will require a significant amount of resources, but it is expected to a huge value to the society, particularly over time.

8. Conclusions and recommendations

8.1. Overarching TORs 1–4

8.1.1. Overarching conclusions

- There is a general consensus among stakeholders that the characteristics of a healthy managed honeybee colony are:
  - it has an adequate size, demographic structure and behaviour in relation to the annual life cycle of the colony and the geographical location;
  - it has an adequate production of bee products in relation to the annual life cycle of the colony and the geographical location;
  - it provides pollination services.
- These three characteristics of a healthy managed honeybee colony have been used to design a hierarchical approach that resulted in the generation of the HEALTHY-B toolbox, which provides guidance on:
  - which indicators, factors and methods could be used to assess bee health;
  - the design of field surveys related to bee health;
  - the analysis of bee health data.

  The toolbox could be consulted by everybody involved in assessing bee health of managed honeybees and aims to facilitate harmonised data collections to assist data analysis and comparisons at regional, national and European level, without imposing a framework that is too rigid.
- The colony attributes 'queen presence and performance', 'demography of the colony', 'in-hive products' and 'disease, infection and infestation' could be measured under field conditions across the EU, but efforts are required to implement these in a harmonised manner.
- The colony attribute 'behaviour and physiology' is difficult to measure in field surveys and the available technology is currently restricted to experimental studies, except the detection of atypical honeybee behaviour.
- Analysing the RPU, particularly land cover/use, of a honeybee colony is very important when assessing its health status, but it currently lacks the tools that could be used at apiary level in field surveys across the EU.
- Data on 'beekeeping management practices' and 'environmental drivers' can be collected via questionnaires and available databases, respectively.
- For 'colony outputs', provisioning services can be analysed mainly for harvested honey, whereas technical limitations hamper the assessment of regulating services in field surveys across the EU.
- The identification of indicators and factors presented in conceptual mind maps and the listed methods suitable for implementation in field surveys across the EU can be considered as tools that could be used to assess bee health and guide harmonisation of data collection and reporting across the EU.
- The specific objectives of a field survey at the national or European level should guide the selection of which indicators, factors and methods from the HEALTHY-B toolbox to use in data collection.
- Integrating multiple attributes of honeybee health, for instance, via a Health Status Index, is required to support a holistic assessment.
- Intensive data collection at a few places across Europe is required to develop a Health Status Index and to calibrate and test risk assessment models. In addition, an epidemiological study involving many apiaries across the EU is necessary to provide complementary information to analyse the relative importance of different stressors (factors), which could then be incorporated in the Health Status Index and/or models. Laboratory data obtained from experimental studies with restricted variability should be used to interpret and complement field data.
- The quality of the collected data determines directly the quality of a bee health analysis. A continuous assessment of data quality is required.

- The benefits to the different stakeholders of applying the toolbox include: harmonisation of data collection/reporting, more efficient use of data collected across the EU, beekeeper involvement in bee health assessments, basis to develop (online) tools that are mutually beneficial to beekeepers, scientists and risk assessors/managers.

8.1.2. Overarching recommendations

- During data collection for assessing bee health in a holistic manner, it is recommended to measure and report indicators and factors at least three times during a year: after winter (e.g. 1–2 weeks after bees start foraging, but before the first big nectar flow), during summer (peak of the active season) and before winter (when the colony is preparing for winter). The timing and frequency of data collection will be determined by the objectives of the field survey.
- It is recommended to involve two people in collecting data on the indicators and factors from a given hive: one to perform the actions and the second to assist with handling and to take notes.
- Developing detailed protocols and training the beekeepers and bee inspectors involved in data collection and reporting is essential to facilitate a harmonised implementation in the field.
- The validation of several tools is required to maximise the precision and accuracy of collected data, in particular if quantitative data are required (e.g. for a model). Several systems are currently under development and/or validation to measure bee health in an automated, smart phone-based and/or remote manner. These initiatives should be positively encouraged because they are considered to have huge potential and in a few years might change data collection.
- Some Member States have detailed databases on bee health (national residue monitoring data, land use/cover, etc.). Efforts are required to improve their accessibility, harmonisation and assure validation of the data included in these databases.
- It is recommended that common terminology (as provided in this scientific opinion) be used to facilitate the interconnection and implementation of similar databases across the EU.
- Continued interaction between the Member State organisations, the EURL and EFSA is required to further facilitate harmonisation of data collection and reporting, to achieve a more efficient use of the data currently collected by many initiatives throughout the EU.
- The development of a sustainable online platform is required to facilitate citizen (i.e. beekeeper) involvement in data provision to the scientific community and subsequently to provide feedback on colony health and production to beekeeper societies. Such a system already exists in other parts of the world and preliminary efforts have been undertaken at the Member State and the European level.
- It is recommended that any analysis of bee health starts with a definition of the goals and purpose of the analysis, before working backwards to the analysis approach and data collection effort needed to achieve those goals.
- Landscape modelling approaches should be further developed for characterisation of the RPU, specifically in the context of bee health. In particular, interactions between the different external drivers and colony attributes need to be better understood, prioritised and quantified. Understanding the precise use of chemical control products in relation to land use is also very important.
- To assess the effects of bee health on ecosystem services, their quantification is recommended, in order to further explore the possible use of a similar framework as developed by the EFSA Plant Health Panel in its guidance document on environmental risk assessment.

8.2. TOR1: Identification of the colony attributes, external drivers and colony outputs

8.2.1. TOR1-specific conclusions

- An examination of the health status of a honeybee colony should consider three overarching concepts: colony attributes, external drivers and colony outputs.
- Queen presence and performance', 'behaviour and physiology', 'demography of the colony', 'in-hive products' and 'disease, infection and infestation' have been identified as the five colony attributes.
- Environmental drivers, RPU and BMP are considered to be the three key external drivers.

- Two honeybee colony outputs have been identified: (i) pollination services, in terms of regulating ecosystem services (= regulating service); and (ii) the products harvested by the beekeeper, the hive rental service and the live honeybees extracted from the colony in terms of provisioning ecosystem services (= provisioning service).

8.3. TOR2: Identification of indicators and factors relevant to measuring colony attributes, external drivers and colony outputs
TOR3: Methods and tools to measure indicators and factors relevant to measuring colony attributes, external drivers and colony outputs

8.3.1. Specific conclusions and recommendations on 'colony attributes'

- The attribute 'queen presence and performance' can be assessed in field surveys across the EU via the indicators 'presence of a queen' and 'potential fecundity'.
- The method suggested to analyse the 'potential fecundity' of a queen is visual identification of the presence of all stages of worker brood.
- The method suggested to analyse the 'presence of a queen' is visual verification by checking through combs and on the walls of the hive.
- Labelling of the queen is recommended, among others, to analyse 'queen longevity' and 'natural queen replacement'. Record keeping is the only method to then assess her longevity by calculating her age and to assess the rate of natural queen replacement (supersedure) over 2 years.
- Beekeepers should note when they replace a queen, especially if in the habit of replacing the queen regularly, regardless of her performance. 'Queen longevity' and 'natural queen replacement' become less informative variables if beekeepers regularly replacing queens themselves.
- The attribute 'demography' can be assessed in field surveys across the EU via the indicators 'colony size', 'dead bees' and 'brood'.
- The method suggested to analyse the 'colony size' is visual estimation of the comb surfaces covered by bees.
- The method suggested to analyse 'brood' is visual estimation of the comb surfaces covered by brood cells.
- The method suggested to analyse 'dead bees' is visual inspection to assess the presence of an unexpectedly high number of dead bees in the hive or in its vicinity.
- The variable 'colony mortality rate' could provide very relevant information to assess the health status of a honeybee colony, but is currently considered feasible only in a research setting.
- The attribute 'in-hive products' can be assessed in field surveys across the EU via the indicators 'bee bread amount', 'bee bread chemical control contamination', 'amount of honey, nectar and honeydew in the nest' and 'wax chemical control contamination'.
- The method suggested to analyse 'bee bread amount' is visual estimation of the number of equivalent combs containing bee bread (Liebefeld estimation method).
- The method suggested to analyse 'honey amount in the nest' is visual estimation of the equivalent number of combs containing honey in the nest (Liebefeld estimation method).
- The method suggested to analyse 'pesticide contamination' in bee bread or wax is multiresidue analysis with a low LOD and LOQ, also taking into account the toxicity (including sub lethal) of the compounds for the bees.
- The attribute 'behaviour and physiology' can be assessed in field surveys across the EU via the indicator 'atypical worker behaviour', which may require training and will be restricted to explicit cases.
- The suggested method to assess 'atypical worker behaviour' inside and/or in the vicinity of the hive (2 m around the hive, including the entrance), is visual identification of atypical worker and queen behaviour.
- The variable 'colony foraging rate' could provide very relevant information to assess the health status of a honeybee colony, but is currently considered feasible only in a research context. However, an unexpected absence of foraging can be observed by a beekeeper and should be reported as abnormal behaviour.

- The attribute 'disease', can be assessed in field surveys across the EU via the indicator 'clinical signs'.
- When clinical signs are observed, it is recommended that the causative agent be identified.
- The attribute 'infection and infestation' can be assessed in field surveys across the EU via systematic analysis of the indicators '*Varroa destructor* infestation' and '*Paenibacillus larvae* infection'. Additional pathogens, pests and/or predators could be included within a given region depending on their regional occurrence. The suggested method to analyse 'clinical signs' is visual inspection of the colony.
- The suggested method to analyse the level of '*Varroa destructor* infestation' is by capturing *Varroa* mites using a sticky trap natural fall (in the hive) or counting the number of mites dislodged with alcohol (on adult bees).
- The suggested methods to analyse '*Paenibacillus larvae* infection' in the hive (in the absence of clinical signs) is by identification of the species *P. larvae* through PCR on honey, adult bees and/or debris.
- Providing data on infection with other pathogens and/or pests has a lower priority for inclusion in field surveys across the EU, although it might be included in specific areas where they are present.
- Identification of *Tropilaelaps* spp. and *Aethina tumida* should be reported because these are notifiable exotic pests.

8.3.2. Specific conclusions and recommendations on 'external drivers'

- The 'RPU' can be assessed in field surveys across the EU by characterising and describing the 'land cover/use' and measuring the floral resources in visited by the honeybees of a colony.
- The 'environmental drivers' can be assessed in field surveys across the EU only via the factors 'temperature', 'relative humidity', 'solar radiation', 'total precipitation', 'climate type' and 'snow cover'.
- The 'beekeeping management practices' can be assessed in field surveys across the EU via the factors 'beekeeping category', 'beekeeper experience', 'location of the apiary', 'introduction of a queen bee', 'change in the number of workers', 'production type of the colony', 'migration activity', 'chemical control', 'replacement of combs with brood', 'replacement of combs with feed sources' and 'supplementary feeding'.
- It is recommended that a method to assess the factor 'beekeeper experience' and the 'colony management' group of factors in a harmonised manner across the EU be developed and implemented.
- It is recommended to increase the accessibility of existing databases containing data that are relevant (and validated) to assess bee health (e.g. environmental drivers, honey contamination).

8.3.3. Specific conclusions on 'colony outputs'

The amount and quality of the outputs represent the ecosystem service provided by the honeybee colony.

- Assessment of the honeybee products (harvested products, hive rental service and live honeybees) in terms of ecosystem provisioning service can provide information on the overall managed honeybee colony performance.
- Although assessment of the pollination service provided by the managed honeybees in terms of ecosystem regulating service is difficult to perform in field conditions, it is an endpoint of the presented framework. In a multifactorial risk assessment of honeybees, the consequences on the pollination services should be estimated. There is a significant lack of information that quantitatively links pollination services with colony health; however, using modelling approaches, it is possible to link this with other colony attributes and external drivers.

8.4. TOR4: Propose a methodological approach to allow robust and harmonised measurement and comparison of regional bee health status

8.4.1. TOR4-specific conclusions

- Analyses of attributes and indexes complement each other, because the reporting of individual attributes is important to facilitate the understanding and transparency of an index.
- Analyses of bee health can aim to describe, explain or predict individual attributes (possible to observe) or indexes of health (constructed based on several attributes).
- A general methodology to assess bee health should preferably include methods for descriptive, explanatory, predictive and prescriptive analyses of bee health.
- Suitable approaches to analyse bee health can be taken from multivariate analysis, expert-driven classification, causal modelling and process-based modelling.
- Analysis considering causal relations between variables or processes expressing dynamics and variability makes it possible to include theoretical and mechanistic understanding to increase information extracted from the data.
- The limitations of data-driven methods to explain and predict health can be addressed by integrating causal and process-based modelling into the analysis.
- Because there is no gold standard measure of bee health, a model to classify the health status of a honeybee colony must rely on expert-informed rules for classification.
- A health index or a process-based model must be properly validated based on data before used in risk assessment and decision support systems.
- Data collection over several years using validated test methods is required to generate models and/or health indexes. Once these models and indexes are validated, continuous data collection and analysis over time are possible.
- It is possible to adapt the sampling design to target specific external drivers or regions and to better quantify ranges of variability in health over geographical space and time.
- Collection of harmonised data are needed across the Member States to facilitate the future development of an HSI.
- Removing a variable from data collection should be avoided until a validated predictive model has identified that the information carried by the variable is redundant.
- It is possible to quantify uncertainty in assessments, predictions of bee health and in model outputs from causal and process-based models.
- It is possible to classify the health status considering uncertainty expert's derived rules and survey data (e.g. missing values, course data).
- Given large data samples, it is possible to quantify ranges of variability in multivariate data-driven methods.

References

Abou-Shaara H, 2014. The foraging behaviour of honey bees, *Apis mellifera*: a review. Veterinární Medicína, 59, 1–10.

AFSSA (Agence française de sécurité sanitaire des aliments), 2009. *Mortalités, effondrements et affaiblissements des colonies d'abeilles [Weakening, collapse and mortality of bee colonies]*. AFSSA, Paris.

Akyol E, Yeninar H, Korkmaz A and Çakmak I, 2009. An observation study on the effects of queen age on some characteristics of honey bee colonies. Italian Journal of Animal Science, 7, 19–26.

Al Toufailia HM, Amiri E, Scandian L, Kryger P and Ratnieks FLW, 2014. Towards integrated control of *Varroa*: effect of variation in hygienic behaviour among honey bee colonies on mite population increase and deformed wing virus incidence. Journal of Apicultural Research, 53, 555–562.

Al Toufailia HM, Scandian L and Ratnieks F, 2015. Towards integrated control of varroa: 2) comparing application methods and doses of oxalic acid on the mortality of phoretic Varroa destructor mites and their honey bee hosts. Journal of Apicultural Research, 54, 108–120.

Alaux C, Brunet J-L, Dussaubat C, Mondet F, Tchamitchan S, Cousin M, Brillard J, Baldy A, Belzunces LP and Le Conte Y, 2010. Interactions between Nosema microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). Environmental Microbiology, 12, 774–782.

Alekseev VA, 1981. Pollination of tetraploid clover [by wild bees and honeybees, ecologic aspects, USSR]. Pchelovodstvo.

Aliferis KA, Copley T and Jabaji S, 2012. Gas chromatography-mass spectrometry metabolite profiling of worker honey bee (*Apis mellifera* L.) hemolymph for the study of *Nosema ceranae* infection. Journal of Insect Physiology, 58, 1349–1359.
Allen M and Ball B, 1996. The incidence and world distribution of honey bee viruses. Bee World, 77, 141–162.
Al-Tikrity WS, Benton AW, Hillman RC and Clarke WW Jr, 1972. The relationship between the amount of unsealed brood in honeybee colonies and their pollen collection. Journal of Apicultural Research, 11, 9–12.
Amdam GV, Aase A, Seehuus SC, Fondrk MK, Norberg K and Hartfelder K, 2005. Social reversal of immunosenescence in honey bee workers. Experimental Gerontology, 40, 939–947.
Anderson TW, 1958. *Introduction to Multivariate Statistical Analysis*. Wiley, New York, NY, USA.
Anderson K, Sheehan T, Eckholm B, Mott B and Degrandi-Hoffman G, 2011. An emerging paradigm of colony health: microbial balance of the honey bee and hive (*Apis mellifera*). Insectes Sociaux, 58, 431–444.
Anderson KE, Sheehan TH, Mott BM, Maes P, Snyder L, Schwan MR, Walton A, Jones BM and Corby-Harris V, 2013. Microbial ecology of the hive and pollination landscape: bacterial associates from floral nectar, the alimentary tract and stored food of honey bees (Apis mellifera). PLoS ONE, 8, e83125.
Andrianakis I, Vernon IR, McCreesh N, McKinley TJ, Oakley JE, Nsubuga RN, Goldstein M and White RG, 2015. Bayesian history matching of complex infectious disease models using emulation: a tutorial and a case study on HIV in Uganda. PLoS Computational Biology, 11, e1003968.
Anklam E, 1998. A review of the analytical methods to determine the geographical and botanical origin of honey. Food Chemistry, 63, 549–562.
ANSES, 2015. Co-exposition des abeilles aux facteurs de stress. Avis de l'Anses Saisine. Rapport d'expertise collective. 268 pp.
Antúnez K, Martín-Hernández R, Prieto L, Meana A, Zunino P and Higes M, 2009. Immune suppression in the honey bee (*Apis mellifera*) following infection by *Nosema ceranae* (Microsporidia). Environmental Microbiology, 11, 2284–2290.
APENET, 2011. Effects of coated maize seed on honey bees. Report based on results obtained from the second year (2011) activity of the APENET project. Available online: http://www.reterurale.it/apenet
Arai R, Tominaga K, Wu M, Okura M, Ito K, Okamura N, Onishi H, Osaki M, Sugimura Y, Yoshiyama M and Takamatsu D, 2012. Diversity of Melissococcus plutonius from honeybee larvae in Japan and experimental reproduction of European foulbrood with cultured atypical isolates. PLoS ONE, 7, e33708.
Arathi H, Burns I and Spivak M, 2000. Ethology of hygienic behaviour in the honey bee *Apis mellifera* L. (Hymenoptera: Apidae): behavioural repertoire of hygienic bees. Ethology, 106, 365–379.
Arnold A, Liu Y and Abe N, 2007. Temporal causal modeling with graphical granger methods. Proceedings of the 13th ACM SIGKDD International Conference on Knowledge Discovery and Data Mining, San Jose, CA, USA, pp. 66–75.
Aubert M, Ball B, Fries I, Moritz RF, Milani N and Bernardinelli I, 2008. *Virology and the Honey Bee*. Office for Official Publications for the European Communities, Luxembourg.
Bacandritsos N, Granato A, Budge G, Papanastasiou I, Roinioti E, Caldon M, Falcaro C, Gallina A and Mutinelli F, 2010. Sudden deaths and colony population decline in Greek honey bee colonies. Journal of Invertebrate Pathology, 105, 335–340.
Bailey L, 1965. The occurrence of chronic and acute bee paralysis viruses in bees outside Britain. Journal of Invertebrate Pathology, 7, 167–169.
Bailey L, 1967. Acute bee-paralysis virus in adult honey bees injected with sacbrood virus. Virology, 33, 368.
Bailey L, 1976. An iridovirus from bees. Journal of General Virology, 31, 459–461.
Bailey L and Woods RD, 1974. Three previously undescribed viruses from the honey bee. Journal of General Virology, 25, 175–186.
Bailey L and Woods RD, 1977. Two more small RNA viruses from honey bees and further observations on sacbrood and acute bee-paralysis viruses. Journal of General Virology, 37, 175–182.
Bailey L, Ball BV, Carpenter JM and Woods RD, 1980. Small virus-like particles in honey bees associated with chronic paralysis virus and with a previously undescribed disease. Journal of General Virology, 46, 149–155.
Bailey L, Ball BV and Perry JN, 1983. Association of viruses with two protozoal pathogens of the honey bee. Annals of Applied Biology, 103, 13–20.
Bailey L, Gibbs A and Woods R, 1964. Sacbrood virus of the larval honey bee (*Apis mellifera* Linnaeus). Virology, 23, 425–429.
Ball BV, 1999. Paralysis. In: Colin ME, Ball BV, Kilani M (eds.). *Bee disease diagnosis*. Options Mediterannéennes. pp. 81–89.
Ball BV and Allen MF, 1988. The prevalence of pathogens in honey bee (Apis mellifera) colonies infested with the parasitic mite Varroa jacobsoni. Annals of Applied Biology, 113, 237–244.
Ball BV and Bailey L, 1997. Viruses. In: Morse RA, Flottum K (eds). *Honey Bee Pests, Predators, and Diseases*. 3rd Edition. A.L. Root, Medina, OH, USA, pp. 11–32.
Bargańska Ż, Ślebioda M and Namieśnik J, 2014. Determination of pesticide residues in honeybees using modified QuEChERS sample work-up and liquid chromatography-tandem mass spectrometry. Molecules, 19, 2911–2924.
Barker RJ, 1977a. Some carbohydrates found in pollen and pollen substitutes are toxic to honey bees. Journal of Nutrition, 107, 1859–1862.

Barker RJ, 1977b. Considerations in selecting sugars for feeding to honey bees. American Bee Journal, 117, 76–77.
Batra LR, Batra SWT and Bohart GE, 1973. The mycoflora of domesticated and wild bees (Apoidea). Mycopathologia et Mycologia Applicata, 43, 13–44.
Baude M, Kunin WE, Boatman ND, Conyers S, Davies N, Gillespie MA and Memmott J, 2016. Historical nectar assessment reveals the fall and rise of floral resources in Britain. Nature, 530, 85–88.
Becher MA, Grimm V, Thorbek P, Horn J, Kennedy PJ and Osborne JL, 2014. BEEHAVE: a systems model of honeybee colony dynamics and foraging to explore multifactorial causes of colony failure. Journal of Applied Ecology, 51, 470–482.
Bedford T and Cooke RM, 2001. *Probabilistic Risk Analysis: Foundations and Methods*. Cambridge University Press, Cambridge, UK.
Beekman M and Ratnieks FLW, 2000. Long-range foraging by the honey-bee, *Apis mellifera* L. Functional Ecology, 14, 490–496.
BeeNet Project, 2013. 3rd 2013 BeeNet Bulletin. Available online: http://www.reterurale.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/9026
Bencsik M, Le Conte Y, Reyes M, Pioz M, Whittaker D, Crauser D, Delso NS and Newton MI, 2015. Honeybee colony vibrational measurements to highlight the brood cycle. PLoS ONE, 10, e0141926.
Benneyan JC, Lloyd RC and Plsek PE, 2003. Statistical process control as a tool for research and healthcare improvement. Quality and Safety in Health Care, 12, 458–464.
Bhattacharya S, 2007. A simulation approach to Bayesian emulation of complex dynamic computer models. Bayesian Analysis, 2, 783–815.
Bigio G, Schürch R and Ratnieks FLW, 2013. Hygienic behavior in honey bees (Hymenoptera: Apidae): effects of brood, food, and time of the year. Journal of Economic Entomology, 106, 2280–2285.
Blacquière T and van der Steen J, 2006. Diagnosis and control of American foulbrood disease of honey bees in the Netherlands. Proceedings of the Section for Experimental and Applied Entomology – Netherlands Entomological Society, 17, 119–121.
Blacquière T, Smagghe G, Van Gestel CA and Mommaerts V, 2012. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. Ecotoxicology, 21, 973–992.
Blanchard P, Ribière M, Celle O, Lallemand P, Schurr F, Olivier V, Iscache AL and Faucon JF, 2007. Evaluation of a real-time two-step RT-PCR assay for quantitation of chronic bee paralysis virus (CBPV) genome in experimentally-infected bee tissues and in life stages of a symptomatic colony. Journal of Virological Methods, 141, 7–13.
Blanchard P, Olivier V, Iscache A-L, Celle O, Schurr F, Lallemand P and Ribière M, 2008a. Improvement of RT-PCR detection of chronic bee paralysis virus (CBPV) required by the description of genomic variability in French CBPV isolates. Journal of Invertebrate Pathology, 97, 182–185.
Blanchard P, Schurr F, Celle O, Cougoule N, Drajnudel P, Thiery R, Faucon J-P and Ribière M, 2008b. First detection of Israeli acute paralysis virus (IAPV) in France, a dicistrovirus affecting honeybees (*Apis mellifera*). Journal of Invertebrate Pathology, 99, 348–350.
Blanchard P, Schurr F, Olivier V, Celle O, Antùnez K, Bakonyi T and Koeglberger H, 2009. Phylogenetic analysis of the RNA-dependent RNA polymerase (RdRp) and a predicted structural protein (pSP) of the Chronic bee paralysis virus (CBPV) isolated from various geographic regions. Virus Research, 144, 334–338.
Blanchard P, Regnault J, Schurr F, Dubois E and Ribière M, 2012. Intra-laboratory validation of chronic bee paralysis virus quantitation using an accredited standardised real-time quantitative RT-PCR method. Journal of Virological Methods, 180, 26–31.
Blanchard P, Carletto J, Siede R, Schurr F, Thiéry R and Ribière M, 2014a. Identification of Kashmir bee virus in France using a new RT-PCR method which distinguishes closely related viruses. Journal of Virological Methods, 198, 82–85.
Blanchard P, Guillot S, Antùnez K, Köglberger H, Kryger P, de Miranda JR, Franco S, Chauzat MP, Thiéry R and Ribière M, 2014b. Development and validation of a real-time two-step RT-qPCR TaqMan® assay for quantitation of Sacbrood virus (SBV) and its application to a field survey of symptomatic honey bee colonies. Journal of Virological Methods, 197, 7–13.
Blaschon B, Guttenberger H, Hrassnigg N and Crailsheim K, 1999. Impact of bad weather on the development of the broodnest and pollen stores in a honeybee colony (Hymenoptera: Apidae). Entomologia Generalis, 24, 49–60.
Bock A, Sparks TH, Estrella N, Jee N, Casebow A, Schunk C, Leuchner M and Menzel A, 2014. Changes in first flowering dates and flowering duration of 232 plant species on the island of Guernsey. Global Change Biology, 20, 3508–3519.
Bogdanov S, 2006. Contaminants of bee products. Apidologie, 37, 1–18.
Bollen KA, 1989. *Structural Equations with Latent Variables*.Wiley, New York, NY, USA.
Boncristiani H, Underwood R, Schwarz R, Evans JD, Pettis J and vanEngelsdorp D, 2012. Direct effect of acaricides on pathogen loads and gene expression levels in honey bees *Apis mellifera*. Journal of Insect Physiology, 58, 613–620.
Bos MM, Veddeler D, Bogdanski AK, Klein A-M, Tscharntke T, Steffan-Dewenter I and Tylianakis JM, 2007. Caveats to quantifying ecosystem services: fruit abortion blurs benefits from crop pollination. Ecological Applications, 17, 1841–1849.

Botía C, Martín-Hernández R, Garrido-Bailón E, González-Porto A, Martínez-Salvador A, De La Rúa P, Meana A and Higes M, 2012. The growing prevalence of Nosema ceranae in honey bees in Spain, an emerging problem for the last decade. Research in Veterinary Science, 93, 150–155.
Bradstreet RB, 1954. Kjeldahl Method for Organic Nitrogen. Analytical Chemistry, 26, 185–187. Available from http://dx.doi.org/10.1021/ac60085a028
Branco MR, Kidd NA and Pickard RS, 2006. A comparative evaluation of sampling methods for Varroa destructor (Acari: Varroidae) population estimation. Apidologie, 37, 452.
Breed MD, Williams DB and Queral A, 2002. Demand for task performance and workforce replacement: undertakers in honeybee, *Apis mellifera*, colonies. Journal of Insect Behavior, 15, 319–329.
British Columbia Ministry of Agriculture. 2012. "Bee Behaviour During Foraging." Apiculture Factsheet. Available at http://www.agf.gov.bc.ca/apiculture/factsheets/111_foraging.htm [Accessed: 23 May 2015]
Brodschneider R and Crailsheim K, 2010. Nutrition and health in honey bees. Apidologie, 41, 278–294.
Brodschneider R, Hrassnigg N, Vollmann J, Petz M, Riessberger-Gallé U and Crailsheim K, 2007. Liquid nutrition within a honeybee colony–who feeds. Apidologie, 38, 492.
Brodschneider R, Moosbeckhofer R and Crailsheim K, 2010. Surveys as a tool to record winter losses of honey bee colonies: a two-year case study in Austria and South Tyrol. Journal of Apicultural Research, 49, 23.
Bromenshenk J, Gudatis J, Carlson S, Thomas J and Simmons M, 1991. Population dynamics of honey bee nucleus colonies exposed to industrial pollutants. Apidologie, 22, 359–369.
Bruni I, Galimberti A, Caridi L, Scaccabarozzi D, De Mattia F, Casiraghi M and Labra M, 2015. A DNA barcoding approach to identify plant species in multiflower honey. Food Chemistry, 170, 308–315.
Büchler R, Berg S and Le Conte Y, 2010. Breeding for resistance to *Varroa destructor* in Europe. Apidologie, 41, 393–408.
Büchler R, Andonov S, Bienefeld K, Costa C, Hatjina F, Kezic N, Kryger P, Spivak M, Uzunov A and Wilde J, 2013. Standard methods for rearing and selection of *Apis mellifera* queens. Journal of Apicultural Research, 52, 1–30.
Büchler R, Costa C, Hatjina F, Andonov S, Meixner MD, Le Conte Y, Uzunov A, Berg S, Bienkowska M, Bouga M, Drazic M, Dyrba W, Kryger P, Panasiuk B, Pechhacker H, Petrov P, Kezić N, Korpela S and Wilde J 2014. The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera* L. colonies in Europe. Journal of Apicultural Research, 53, 205–214.
Budge GE, Barrett B, Jones B, Pietravalle S, Marris G, Chantawannakul P, Thwaites R, Hall J, Cuthbertson AG and Brown MA, 2010. The occurrence of Melissococcus plutonius in healthy colonies of Apis mellifera and the efficacy of European foulbrood control measures. Journal of Invertebrate Pathology, 105, 164–170.
Budge GE, Pietravalle S, Brown M, Laurenson L, Jones B, Tomkies V and Delaplane KS, 2015. Pathogens as predictors of honey bee colony strength in England and Wales. PLoS ONE, 10, e0133228.
Burdock GA, 1998. Review of the biological properties and toxicity of bee propolis (propolis). Food and Chemical Toxicology, 36, 347–363.
Burrill RM and Dietz A, 1981. The response of honey bees to variations in solar radiation and temperature. Apidologie, 12, 319–328.
Bush AO, Fernández JC, Esch GW and Seed JR, 2001. *Parasitism: The Diversity and Ecology of Animal Parasites*. Cambridge University Press, Cambridge, UK.
Butler CG, 1975. The honeybee colony. Life history. In: Dadant and Sons (eds.). *The Hive and the Honey Bee*. Hamilton, IL, USA.
Cameletti M, Lindgren F, Simpson D and Rue H, 2013. Spatio-temporal modeling of particulate matter concentration through the SPDE approach. AStA Advances in Statistical Analysis, 97, 109–131.
Cameron AC and Trivedi PK, 2005. *Microeconometrics: Methods and Applications*. Cambridge University Press, New York.
Campos MG, Bogdanov S, de Almeida-Muradian LB, Szczesna T, Mancebo Y, Frigerio C and Ferreira F, 2008. Pollen composition and standardisation of analytical methods. Journal of Apicultural Research, 47, 154–161.
Carletto J, Blanchard P, Gauthier A, Schurr F, Chauzat M-P and Ribière M, 2013. Improving molecular discrimination of *Nosema apis* and *Nosema ceranae*. Journal of Invertebrate Pathology, 113, 52–55.
Carletto J, Gauthier A, Regnault J, Blanchard P, Schurr F and Ribière-Chabert M, 2010. Detection of main honey bee pathogens by multiplex PCR. Euro Reference, 4, 13–15.
Carreck NL and Williams IH, 2002. Food for insect pollinators on farmland: insect visits to flowers of annual seed mixtures. Journal of Insect Conservation, 6, 13–23.
Carvell C, Meek WR, Pywell RF, Goulson D and Nowakowski M, 2007. Comparing the efficacy of agri-environment schemes to enhance bumble bee abundance and diversity on arable field margins. Journal of Applied Ecology, 44, 29–40.
Cepero A, Higes M, Martinez-Salvador A, Meana A and Martin-Hernandez R, 2014a. A two year national surveillance for *Aethina tumida* reflects its absence in Spain. BMC Research Notes, 7, 878.
Cepero A, Ravoet J, Gomez-Moracho T, Bernal JL, Del Nozal MJ, Bartolome C, Maside X, Meana A, Gonzalez-Porto AV, de Graaf DC, Martin-Hernandez R and Higes M, 2014b. Holistic screening of collapsing honey bee colonies in Spain: a case study. BMC Research Notes, 7, 649.

Cersini A, Antognetti V, Conti R, Velletrani F and Formato G, 2015. First PCR isolation of *Crithidia mellificae* (Euglenozoa: Trypanosomatidae) in *Apis mellifera* (Hymenoptera: Apidae) in Italy. Fragmenta Entomologica, 47, 45–49.

Cersini A, Bellucci V, Lucci S, Mutinelli F, Granato A, Porrini C and Formato G, 2013. First isolation of Kashmir bee virus (KBV) in Italy. Journal of Apicultural Research, 52, 54–55.CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS). Available online: http://ccafs-climate.org/data/

Chaimanee V, Chantawannakul P, Chen Y, Evans JD and Pettis JS, 2012. Differential expression of immune genes of adult honey bee (*Apis mellifera*) after inoculated by *Nosema ceranae*. Journal of Insect Physiology, 58, 1090–1095.

Chaskopoulou A, Thrasyvoulou A, Goras G, Tananaki C, Latham MD, Kashefi J, Pereira RM and Koehler PG, 2014. Nontarget effects of aerial mosquito adulticiding with water-based unsynergized pyrethroids on honey bees and other beneficial insects in an agricultural ecosystem of North Greece. Journal of Medical Entomology, 51, 720–724.

Chauzat M-P, Martel A-C, Blanchard P, Clement M-C, Schurr F, Lair C, Ribière M, Wallner K, Rosenkranz P and Faucon J-P, 2010. A case report of a honey bee colony poisoning. Journal of Apicultural Research, 49, 113–115.

Chauzat MP, Martel AC, Cougoule N, Porta P, Lachaize J, Zeggane S, Aubert M, Carpentier P and Faucon JP, 2011. An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera: Apidae) to monitor pesticide presence in continental France. Environmental Toxicology and Chemistry, 30, 103–111.

Chauzat M-P, Cauquil L, Roy L, Franco S, Hendrikx P and Ribière-Chabert M, 2013. Demographics of the European apicultural industry. PLoS ONE, 8, e79018.

Chauzat P, Laurent M, Rivière M-P, Saugeon C, Hendrikx P and Ribière-Chabert M and Pathology Unit H, 2014. A pan-European epidemiological study on honey bee colony losses 2012–2013. Rapport Technique. European Union Reference Laboratory for Honeybee Health, Brussels.

Chen YP, Higgins JA and Feldlaufer MF, 2005. Quantitative real-time reverse transcription-PCR analysis of deformed wing virus infection in the honeybee (Apis mellifera L.). Applied and Environmental Microbiology, 71, 436–441.

Chilès JP and Delfiner P, 1999. *Multivariate Methods. Geostatistics: Modeling Spatial Uncertainty*. John Wiley & Sons pp. 299–385.

Ciglenečki UJ and Toplak I, 2012. Development of a real-time RT-PCR assay with TaqMan probe for specific detection of acute bee paralysis virus. Journal of Virological Methods, 184, 63–68.

Clark TB, 1977. *Spiroplasma* sp., a new pathogen in honey bees. Journal of Invertebrate Patholoogy, 29, 112–113.

Clark TB, 1978. Honey bee Spiroplasmosis – new problem for beekeepers. American Bee Journal, 118, 18–19.

Clermont A, Eickermann M, Kraus F, Hoffmann L and Beyer M, 2015. Correlations between land covers and honey bee colony losses in a country with industrialized and rural regions. Science of the Total Environment, 532, 1–13.

Coineau Y and Fernandez N, 2007. La nosémose. In: Atlantica (eds.), *L'abeille mellifère – Maladies, parasites et autres ennemis*. Collection Atlantisciences, Atlantica. 130 pp.

Collins A, 2000. Relationship between semen quality and performance of instrumentally inseminated honey bee queens. Apidologie, 31, 421–429.

Collins AM and Kubasek KJ, 1982. Field test of honey bee (Hymenoptera: Apidae) colony defensive behavior. Annals of the Entomological Society of America, 75, 383–387.

Cook SM, Awmack CS, Murray DA and Williams IH, 2003. Are honey bees' foraging preferences affected by pollen amino acid composition? Ecological Entomology, 28, 622–627.

Cooke CM, Shaw G, Lester JN and Collins CD, 2004. Determination of solid-liquid partition coefficients (Kd) for diazinon, propetamphos and cis-permethin: implications for sheep dip disposal. Science of The Total Environment, 329, 197–213. doi: 10.1016/j.scitotenv.2004.02.021

Corbella E and Gonçalves LS, 1982. Relationship between weight at emergence, number of ovarioles and spermathecal volume of africanized honey bee queens (apis-mellifera l). Revista Brasileira de Genetica, 5, 835–840.

Corby-Harris V, Maes P and Anderson KE, 2014. The bacterial communities associated with honey bee (Apis mellifera) foragers. PLoS ONE, 9, e95056.

Core A, Runckel C, Ivers J, Quock C, Siapno T, DeNault S, Brown B, DeRisi J, Smith CD and Hafernik J, 2012. A new threat to honey bees, the parasitic phorid fly *Apocephalus borealis*. PLoS ONE, 7, e29639. doi: 10.1371/journal.pone.0029639

Cornman RS, Tarpy DR, Chen Y, Jeffreys L, Lopez D, Pettis JS and Evans JD, 2012. Pathogen webs in collapsing honey bee colonies. PLoS ONE, 7, e43562.

Costa C, Buechler R, Berg S, Bienkowska M, Bouga M, Bubalo D, Charistos L, Le Conte Y, Drazic M, Dyrba W, Fillipi J, Hatjina F, Ivanova E, Kezic N, Kiprijanovska H, Kokinis M, Korpela S, Kryger P, Lodesani M, Meixner M, Panasiuk B, Pechhacker H, Petrov P, Oliveri E, Ruottinen L, Uzunov A, Vaccari G and Wilde J, 2012. A Europe-wide experiment for assessing the impact of genotype–environment interactions on the vitality and performance of honey bee colonies: experimental design and trait evaluation. Journal of Apicultural Science, 56, 147–158.

Couvillon MJ, Fensome KA, Quah SKL and Schürch R, 2014a. Summertime blues: August foraging leaves honey bees empty-handed. Communicative and Integrate Biology, 7, e28821.

Couvillon MJ, Schürch R and Ratnieks FLW, 2014b. Dancing bees communicate a foraging preference for rural lands under High Level Agri-Environment Schemes. Current Biology, 24, 1212–1215.

Couvillon MJ, Riddell Pearce FC, Accleton C, Fensome KA, Quah SKL, Taylor EL and Ratnieks FLW, 2014c. Honey bee foraging distance depends on month and forage type. Apidologie, 46, 1–10.
Couvillon MJ, Schürch R and Ratnieks FLW, 2014d. Waggle dance distances as integrative indicators of seasonal foraging challenges. PLoS ONE, 9, e93495.
Cox LA, 2012. *Improving Risk Analysis*. Springer, New York, NY, USA.
Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA, Quan P-L, Briese T, Hornig M and Geiser DM, 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. Science, 318, 283–287.
Crailsheim K, Stabentheiner A, Hrassnigg N and Leonhard B, 1999. Oxygen consumption at different activity levels and ambient temperatures in isolated honeybees (Hymenoptera: Apidae). Entomologia Generalis, 24, 1–12.
Crane E, 1975. *Honey: A Comprehensive Survey*. Heinemann, London, UK.
Cremer S, Armitage SA and Schmid-Hempel P, 2007. Social immunity. Current Biology, 17, R693–R702.
Cuddington K, Fortin M-J, Gerber LR, Hastings A, Liebhold A, O'Connor M and Ray C, 2013. Process-based models are required to manage ecological systems in a changing world. Ecosphere, 4, 1–12.
Cuevas-Glory LF, Pino JA, Santiago LS and Sauri-Duch E, 2007. A review of volatile analytical methods for determining the botanical origin of honey. Food Chemistry, 103, 1032–1043.
Cutler GC, Scott-Dupree CD and Drexler DM, 2014. Honey bees, neonicotinoids and bee incident reports: the Canadian situation. Pest Management Science, 70, 779–783.
Dafni A, Kevan PG and Husband BC. *Practical pollination biology. Practical pollination biology*. Enviroquest, Cambridge, ON, Canada
Dainat B, Evans JD, Chen YP, Gauthier L and Neumann P, 2012a. Predictive markers of honey bee colony collapse. PLoS ONE. 7, doi: 10.1371/journal.pone.0032151
Dainat B, Evans JD, Chen YP, Gauthier L and Neumann P, 2012b. Dead or alive: deformed wing virus and Varroa destructor reduce the life span of winter honeybees. Applied and Environmental Microbiology, 78, 981–987.
Danka RG and Beaman LD, 2007. Flight activity of USDA–ARS Russian honey bees (Hymenoptera: Apidae) during pollination of lowbush blueberries in Maine. Journal of Economic Entomology, 100, 267–272.
Danka RG, De Guzman LI, Rinderer TE, Sylvester HA, Wagener CM, Bourgeois AL, Harris JW and Villa JD, 2012. Functionality of *Varroa*-resistant honey bees (Hymenoptera: Apidae) when used in migratory beekeeping for crop pollination. Journal of Economic Entomology, 105, 313–321.
Danka RG, Harris JW, Villa JD and Dodds GE, 2013. Varying congruence of hygienic responses to *Varroa destructor* and freeze-killed brood among different types of honeybees. Apidologie, 44, 447–457.
Davis RS, Peterson RKD and Macedo PA, 2007. An ecological risk assessment for insecticides used in adult mosquito management. Integrated Environmental Assessment and Management, 3, 373–382.
Decourtye A and Devillers J, 2010. Ecotoxicity of neonicotinoid insecticides to bees. In: Thany SH (ed). *Insect Nicotinic Acetylcholine Receptors*. Springer, New York, NY, USA. pp. 85–95.
DeGrandi-Hoffman G, Chen Y, Huang E and Huang MH, 2010. The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.). Journal of Insect Physiology, 56, 1184–1191.
DeGrandi-Hoffman G, Ahumada F, Zazueta V, Chambers M, Hidalgo G and Watkins deJong E, 2016. Population growth of *Varroa destructor* (Acari: Varroidae) in honey bee colonies is affected by the number of foragers with mites. Experimental and Applied Acarology, 69, 21–34.
Delaplane KS and Mayer NF, 2000. *Crop Pollination by Bees*. CABI, Wallingford, UK.
Delaplane KS, Dag A, Danka RG, Freitas BM, Garibaldi LA, Goodwin RM and Hormaza JI, 2013a. Standard methods for pollination research with Apis mellifera. Journal of Apicultural Research, 52, 1–28.
Delaplane KS, van der Steen J and Guzman-Novoa E, 2013. Standard methods for estimating strength parameters of Apis mellifera colonies. Journal of Apicultural Research, 52, 1–12.
Delaplane KS, van der Steen J and Guzman-Novoa E, 2013b. Standard methods for estimating strength parameters of *Apis mellifera* colonies. Journal of Apicultural Research, 52, 1–12. doi: 10.3896/IBRA.1.52.1.03
Deloitte, 2013. Evaluation of Measures for the Apicultural Sector. Available online: ec.europa.eu/agriculture/evaluation/market-and-income. . ./apiculture-2013_en.htm
Di Pasquale G, Salignon M, Le Conte Y, Belzunces LP, Decourtye A, Kretzschmar A, Suchail S, Brunet J-L and Alaux C, 2013. Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter? PLoS ONE, 8, e72016.
Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, Nazzi F, Gargiulo G and Pennacchio F, 2013. Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. Proceedings of the National Academy of Sciences of the United States of America, 110, 18466–18471.
Dietemann V, Nazzi F, Martin SJ, Anderson DL, Locke B, Delaplane KS, Wauquiez Q, Tannahill C, Frey E and Ziegelmann B, 2013. Standard methods for *Varroa* research. Journal of Apicultural Research, 52, 1–54.
Dietz A, 1975. Nutrition of the adult honey bee. The Hive and the Honey bee, 740, 125–156
Dobbelaere W, De Graaf D and Peeters J, 2001. Development of a fast and reliable diagnostic method for American foulbrood disease (Paenibacillus larvae subsp. larvae) using a 16S rRNA gene based PCR. Apidologie, 32, 363–370.

Dobrynin N, Colombo M and Eördegh F, 2013. A comparative study of diagnostic methods for detection of *Varroa destructor* infestation level in honey bee (*Apis mellifera*) colonies. Journal Acarina, 21, 3–16.
Döke MA, Frazier M and Grozinger CM, 2015. Overwintering honey bees: biology and management. Current Opinion in Insect Science, 10, 185–193.
Dorer B, 2014. ESS Round 7 Translation Guidelines. European Social Survey. 49 pp. Available online: https://www.europeansocialsurvey.org/docs/methodology/ESS_R7_Translation_Guidelines_FINAL.pdf
Dornhaus A, Klügl F, Oechslein C, Puppe F and Chittka L, 2006. Benefits of recruitment in honey bees: effects of ecology and colony size in an individual-based model. Behavioral Ecology, 2006, doi:10.1093/beheco/arj036 Advance Access publication 18 January.
Doublet V, Labarussias M, Miranda JR, Moritz RF and Paxton RJ, 2015. Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. Environmental Microbiology, 17, 969–983.
Doul KM, 1976. The effects of different humidities on the hatching of the eggs of honeybees. Apidologie, 7, 61–66.
Downs SG, Ratnieks FL, Badcock NS and Mynott A, 2001. Honeybee guards do not use food-derived odors to recognize non-nest mates: a test of the Odor Convergence hypothesis. Behavioral Ecology, 12, 47–50.
Dungan RJ, Beggs JR and Wardle DA, 2004. A simple gravimetric technique for estimating honeydew or nectar production. New Zealand Journal of Ecology, 28, 283–288.
Dussaubat C, Brunet J-L, Higes M, Colbourne JK, Lopez J, Choi JH, Martin-Hernandez R, Botías C, Cousin M, McDonnell C, Bonnet M, Belzunces LP, Moritz RFA, Le Conte Y and Alau C, 2012. Gut pathology and responses to the microsporidium Nosema ceranae in the honey bee *Apis mellifera*. PLoS ONE, 7, e37017.
Dussaubat C, Sagastume S, Gómez-Moracho T, Botías C, García-Palencia P, Martin-Hernandez R, Le Conte Y and Higes M, 2013. Comparative study of *Nosema ceranae* (Microsporidia) isolates from two different geographic origins. Veterinary Microbiology, 162, 670–678.
EASAC Policy Report 26, 2015. Ecosystem services, agriculture and neonicotinoids. Available online: http://www.easac.eu/home/reports-and-statements/detail-view/article/ecosystem-se.html
Edwards-Murphy F, Magno M, Whelan PM, O'Halloran J and Popovici EM, 2016. b+WSN: Smart beehive with preliminary decision tree analysis for agriculture and honey bee health monitoring. Computers and Electronics in Agriculture, 124, 211–219.
EEA (European Environment Agency), 2014. Technical report on CLC2006 technical guidelines. ISSN 1725-2237 [66 pp].
EFSA (European Food Safety Authority), 2008. Bee Mortality and Bee Surveillance in Europe—a report from the Assessment Methodology Unit in Response to Agence Francaise de Sécurité Alimentaire des Aliments (AFSSA). EFSA Journal 2008;(6)8:154r, 28 pp. doi:10.2903/j.efsa.2008.154r
EFSA (European Food Safety Authority) Panel on Plant Health, 2011. Guidance on the envirnmental risk assessment of plant pests. EFSA Journal 2011;9(12):2460, 121 pp. doi:10.2903/j.efsa.2011.2460
EFSA (European Food Safety Authority), 2012a. Scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 2012;10(5):2668, 275 pp.
EFSA (European Food Safety Authority), 2012b. Statement on the use of animal-based measures to assess the welfare of animals. EFSA Journal 2012;10(6):2767, 29 pp.
EFSA (European Food Safety Authority), 2013. Towards holistic approaches to the risk assessment of multiple stressors in bees. 76 pp.
EFSA (European Food Safety Authority), 2014. Towards an integrated environmental risk assessment of multiple stressors on bees: review of research projects in Europe, knowledge gaps and recommendations. EFSA Journal 2014;12(3):3594, 102 pp. doi: 10.2903/j.efsa.2014.3594
EFSA (European Food Safety Authority), 2015. Survival, spread and establishment of the small hive beetle (*Aethina tumida*). EFSA Journal 2015;13(12):4328, 77 pp. doi:10.2903/j.efsa.2015.4328
EFSA (European Food Safety Authority), 2016a. Workshop 'The health status of a managed honeybee colony'. EFSA supporting publication 2016:EN-1055, 14 pp.
EFSA (European Food Safety Authority), 2016b. A mechanistic model to assess risks to honeybee colonies from exposure to pesticides under different scenarios of combined stressors and factors. EFSA supporting publication 2016: 13(7):EN-1069. 116 pp. doi: 10.2903/sp.efsa.2016.EN-1069
Ellis MB (2008) Homeostasis: Humidity and water relation in honeybee colony. Master's Thesis, University of Pretoria, South Africa.
Ellis JD and Munn PA, 2005. The worldwide health status of honey bees. Bee World, 86, 88–101.
Ellis JD Jr, Rong IH, Hill MP, Hepburn HR and Elzen PJ, 2004. The susceptibility of small hive beetle (*Aethina tumida* Murray) pupae to fungal pathogens. American Bee Journal, 144, 486–488.
vanEngelsdorp D, Caron D, Hayes J, Underwood R, Henson M, Rennich K, Spleen A, Andree M, Snyder R, Lee K and Roccasecca K, 2012. A national survey of managed honey bee 2010–11 winter colony losses in the USA: results from the Bee Informed Partnership. Journal of Apicultural Research, 51, 115–124.
vanEngelsdorp D, Lengerich E, Spleen A, Dainat B, Cresswell J, Baylis K, Nguyen BK, Soroker V, Underwood R and Human H, 2013. Standard epidemiological methods to understand and improve Apis mellifera health. Journal of Apicultural Research, 52, 1–16.

Esposito Vinzi V, Chin WW, Henseler J and Wang W, 2010. *Handbook of Partial Least Squares: Concepts, Methods and Applications*. Springer, Berlin.
EUNIS, 2007. Database of EUNIS habitat classification, revised in 2012. European Environmental Agency. Available online: http://www.eea.europa.eu/themes/biodiversity/eunis/eunis-habitat-classification
Eurostat, 2007. The use of plant protection products in the European Union Data 1992–2003. Available online: http://ec.europa.eu/urostat/documents/3217494/5611788/KS-76-06-669-EN.PDF/36c156f1-9fa9-4243-9bd3-f4c7c3c8286a?version=1.0
Eurostat, 2012. Agri-environmental indicator – consumption of pesticides. Available online: http://ec.europa.eu/eurostat/statistics-explained/index.php/Agri-environmental_indicator_-_consumption_of_pesticides
Eurostat, 2014. Agriculture, forestry and fishery statistics. Available online: http://ec.europa.eu/eurostat/en/web/products-statistical-books/-/KS-FK-14-001
Eurostat, 2016. Land cover, concepts and definitions database. Reference and management of nomenclature. Available online: http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=DSP_GLOSSARY_NOM_DTL_VIEW&StrNom=CODED2&StrLanguageCode=EN&IntKey=16511235&RdoSearch=&TxtSearch=&CboTheme=&IntCurrentPage=1
Evans JD and Lopez DL, 2004. Bacterial probiotics induce an immune response in the honey bee (Hymenoptera: Apidae). Journal of Economic Entomology, 97, 752–756.
Evans JD and Spivak M, 2010. Socialized medicine: individual and communal disease barriers in honey bees. Journal of Invertebrate Pathology, 103, S62–S72.
Evans JD, Aronstein K, Chen Y, Hetru C, Imler JL, Jiang H, Kanost M, Thompson G, Zou Z and Hultmark D, 2006. Immune pathways and defence mechanisms in honey bees *Apis mellifera*. Insect Molecular Biology, 15, 645–656.
Fabian D and Flatt T, 2012. Life history evolution. Nature Education Knowledge, 3, 24.
Fahrenholz L, Lamprecht I and Schricker B, 1989. Thermal investigations of a honey bee colony: thermoregulation of the hive during summer and winter and heat production of members of different bee castes. Journal of Comparative Physiology B, 159, 551–560.
Fakhimzadeh K, Hokkanen H, Sikkilä J, Pirhonen K and Lintula E, 1993. The first survey of *Acarapis woodi* in Finland. Bee World, 74, 129–133.
Fao U, 1999. *Terminology for Integrated Resources Planning and Management*. Food and Agriculture Organization/United Nations Environmental Programme, Rome, Italy/Nairobi, Kenia.
Fell RD and Morse RA, 1984. Emergency queen cell production in the honey bee colony. Insectes Sociaux, 31, 221–237.
Ferrari TE, 2014. Magnets, magnetic field fluctuations and geomagnetic disturbances impair the homing ability of honey bees (*Apis mellifera*). Journal of Apicultural Research, 53, 452–465.
Fewell JH and Winston ML, 1992. Colony state and regulation of pollen foraging in the honey bee, Apis mellifera L. Behavioral Ecology and Sociobiology, 30, 387–393.
Fischer MK and Shingleton AW, 2001. Host plant and ants influence the honeydew sugar composition of aphids. Functional Ecology, 15, 544–550.
Fleche C, Clement M, Zeggane S and Faucon J, 1997. Contamination of bee products and risk for human health: situation in France. Revue Scientifique et Technique (International Office of Epizootics), 16, 609–619.
Flores J, Gil S and Padilla F, 2015. Reliability of the main field diagnostic methods of *Varroa* in honey bee colonies. Archivos de Zootecnia, 64, 161–166.
Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR and Helkowski JH, 2005. Global consequences of land use. Science, 309, 570–574.
Foley K, Fazio G, Jensen AB and Hughes WO, 2014. The distribution of *Aspergillus* spp. opportunistic parasites in hives and their pathogenicity to honey bees. Veterinary Microbiology, 169, 203–210.
Formato G, Giacomelli A, Olivia MA, Aubin L, Glick E, Paldi N and Granato A, 2011. First detection of Israeli acute paralysis virus (IAPV) in Italy. Journal of Apicultural Research, 50, 176–177.
Formicki G, Gren A, Stawaez R, Zysk B and Gal A, 2013. Metal content in honey, propolis, wax, and bee pollen and implications for metal pollution monitoring. Polish Journal of Environmental Studies, 22, 99–106.
Forsgren E, 2010. European foulbrood in honey bees. Journal of Invertebrate Pathology, 103, S5–S9.
Forsgren E and Laugen AT, 2014. Prognostic value of using bee and hive debris samples for the detection of American foulbrood disease in honey bee colonies. Apidologie, 45, 10–20.
Forsgren E, Budge GE, Charrière J-D and Hornitzky MAZ, 2013. Standard methods for European foulbrood research. Journal of Apicultural Research, 52, 1–14.
Francis R and Kryger P, 2012. Single assay detection of Acute bee paralysis virus, Kashmir bee virus and Israeli acute paralysis virus. Journal of Apicultural Science, 56, 137–146.
Francis RM, Kryger P, Meixner M, Bouga M, Ivanova E, Andonov S, Berg S, Bienkowska M, Büchler R and Charistos L, 2014. The genetic origin of honey bee colonies used in the COLOSS Genotype-Environment Interactions Experiment: a comparison of methods. Journal of Apicultural Research, 53, 188–204.
Fresnaye J, 1961. Méthodes d'appréciation des surfaces de couvain dans les colonies d'abeilles. Annales de L'Abeille, 4, 369–376.

Fries I, 1991. Treatment of sealed honey bee brood with formic acid for control of Varroa jacobsoni. American bee Journal (USA).
Fries I, 1993. *Nosema apis* – a parasite in the healthy bee colony. Bee World, 74, 5–19.
Fröhlich B, Tautz J and Riederer M, 2000. Chemometric classification of comb and cuticular waxes of the honeybee *Apis mellifera carnica* Pollm. (Hymenoptera: Apidae). Journal of Chemical Ecology, 26, 123–137.
Fujiyuki T, Takeuchi H, Ono M, Ohka S, Sasaki T, Nomoto A and Kubo T, 2004. Novel insect picorna-like virus identified in the brains of aggressive worker honeybees. Journal of Virology, 78, 1093–1100.
Furgala B, 1975. Chapter XVI; Fall Management and the Wintering of Productive Colonies. In: *The hive and the honeybee*. University of Minnesota, Dadant.
Fyg W, 1964. Anomalies and diseases of the queen honey bee. Annual Review of Entomology, 9, 207–224.
Gafurov A and Bárdossy A, 2009. Cloud removal methodology from MODIS snow cover product. Hydrology and Earth System Sciences, 13, 1361–1373.
Gao Y, Xie H, Lu N and Liang T, 2010. Toward advanced daily cloud-free snow cover and snow water equivalent products from Terra-Aqua MODIS and Aqua AMSR-E measurements. Journal of Hydrology, 385, 23–35.
Garratt MPD, Breeze TD, Boreux V, Fountain MT, McKerchar M, Webber SM, Coston DJ, Jenner N, Dean R, Westbury DB, Biesmeijer JC and Potts SG, 2016. Apple Pollination: Demand Depends on Variety and Supply Depends on Pollinator Identity. PLoS ONE, 11, e0153889.
Garratt MPD, Coston DJ, Truslove CL, Lappage MG, Polce C, Dean R, Biesmeijer JC and Potts SG, 2014. The identity of crop pollinators helps target conservation for improved ecosystem services. Biological Conservation, 169, 128–135.
Garrido-Bailon E, Higes M, Martinez-Salvador A, Antunez K, Botias C, Meana A, Prieto L and Martin-Hernandez R, 2013. The prevalence of the honeybee brood pathogens Ascosphaera apis, Paenibacillus larvae and Melissococcus plutonius in Spanish apiaries determined with a new multiplex PCR assay. Microbial Biotechnology, 6, 731–739. doi: 10.1111/1751-7915.12070
Gary NE and Lorenzen K, 1976. *How to construct and maintain an observation bee hive*. University of California, Leaflet 2853.
Gelman A, Carlin JB, Stern HS and Rubin DB, 2014. *Bayesian Data Analysis* (Vol 2). Chapman and Hall/CRC, Boca Raton, FL, USA.
GEMET (General Multilingual Environmental Thesaurus), Accessed 2016. Available at http://www.eionet.europa.eu/gemet
Gende L, Satta A, Ligios V, Ruiu L, Buffa F, Fernandez N, Churio S, Eguaras M, Fiori M and Floris I, 2011. Searching for an American foulbrood early detection threshold by the determination of *Paenibacillus larvae* spore load in worker honey bees. Bulletin of Insectology, 64, 229–233.
Genersch E, 2010. Honey bee pathology: current threats to honey bees and beekeeping. Applied Microbiology and Biotechnology, 87, 87–97.
Genersch E and Aubert M, 2010. Emerging and re-emerging viruses of the honey bee (*Apis mellifera* L.). Veterinary Research, 41, 54.
Genersch E, Ashiralieva A and Fries I, 2005. Strain-and genotype-specific differences in virulence of Paenibacillus larvae subsp. larvae, a bacterial pathogen causing American foulbrood disease in honeybees. Applied and Environmental Microbiology, 71, 7551–7555.
Genersch E, Von Der Ohe W, Kaatz H, Schroeder A, Otten C, Büchler R, Berg S, Ritter W, Mühlen W and Gisder S, 2010. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie, 41, 332–352.
Giacobino A, Molineri A, Cagnolo NB, Merke J, Orellano E, Bertozzi E, Masciángelo G, Pietronave H, Pacini A and Salto C, 2015. Risk factors associated with failures of *Varroa* treatments in honey bee colonies without broodless period. Apidologie, 46, 573–582.
Gilioli G, Schrader G, Baker RHA, Ceglarska E, Kertész VK, Lövei G ... and van Lenteren JC, 2014. Environmental risk assessment for plant pests: a procedure to evaluate their impacts on ecosystem services. Science of the Total Environment, 468, 475–486.
Gilliam M and Morton HL, 1978. Bacteria belonging to the genus Bacillus isolated from honey bees, Apis mellifera, fed 2, 4-D and antibiotics. Apidologie, 9, 213–222.
Girolami V, Marzaro M, Vivan L, Mazzon L, Greatti M, Giorio C and Tapparo A, 2012. Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication. Journal of Applied Entomology, 136, 17–26.
Gisder S, Hedtke K, Möckel N, Frielitz M-C, Linde A and Genersch E, 2010. Five-year cohort study of Nosema spp. in Germany: does climate shape virulence and assertiveness of Nosema ceranae? Applied and Environmental Microbiology, 76, 3032–3038.
Govan VA, Allsopp MH and Davison S, 1999. A PCR detection method for rapid identification of Paenibacillus larvae. Applied and Environmental Microbiology, 65, 2243–2245.
Grace JB, Anderson TM, Olff H and Scheiner SM, 2010. On the specification of structural equation models for ecological systems. Ecological Monographs, 80, 67–87.

Granberg F, Vicente-Rubiano M, Rubio-Guerri C, Karlsson OE, Kukielka D, et al., 2013. Metagenomic detection of viral pathogens in Spanish honeybees: co-infection by aphid lethal paralysis, Israel acute paralysis and Lake Sinai viruses. PLoS ONE, 8, e57459. doi:10.1371/journal.pone.0057459

Gregorc A, 2012. A clinical case of honey bee intoxication after using coumaphos strips against Varroa destructor. Journal of Apicultural Research, 51, 142–143.

Gregorc A, Evans JD, Scharf M and Ellis JD, 2012. Gene expression in honey bee (*Apis mellifera*) larvae exposed to pesticides and Varroa mites (*Varroa destructor*). Journal of Insect Physiology, 58, 1042–1049.

Grimm V and Railsback SF, 2013. *Individual-Based Modeling and Ecology*. Princeton University Press, Princeton, NJ, USA.

de Groot AP, 1953. Protein and amino acid requirements of the honeybee (*Apis mellifica* L.). Physiologia Comparata et Oecologia, 3, 197–285.

Hagenaars JA, 1993. *Loglinear Models with Latent Variables* (Vol. 94). Sage.

Hagenbucher S, Wäckers FL and Romeis J, 2014. Aphid honeydew quality as a food source for parasitoids is maintained in Bt Cotton. PLoS ONE, 16, e107806.

Hamiduzzaman MM, Guzman-Novoa E, Goodwin PH, Reyes-Quintana M, Koleoglu G, Correa-Benitez A and Petukhova T, 2015. Differential responses of Africanized and European honey bees (*Apis mellifera*) to viral replication following mechanical transmission or *Varroa destructor* parasitism. Journal of Invertebrate Pathology, 126, 12–20.

Harrison JF, Camazine S, Marden JH, Kirkton SD, Rozo A and Yang X, 2001. Mite not make it home: tracheal mites reduce the safety margin for oxygen delivery of flying honeybees. Journal of Experimental Biology, 204, 805–814.

Hartig F, Calabrese JM, Reineking B, Wiegand T and Huth A, 2011. Statistical inference for stochastic simulation models – theory and application. Ecology Letters, 14, 816–827.

Hartig F, Dyke J, Hickler T, Higgins SI, O'Hara RB, Scheiter S and Huth A, 2012. Connecting dynamic vegetation models to data – an inverse perspective. Journal of Biogeography, 39, 2240–2252.

Hasemann L, 1961. How long can spores of American foulbrood live? American Bee Journal, 101, 298–299.

Hastie T, Tibshirani R and Friedman J, 2009. Unsupervised learning. In: *The Elements of Statistical Learning*. Springer, New York, NY, USA, pp. 485–585.

Hatch S, Tarpy DR and Fletcher DJC, 1999. Worker regulation of emergency queen rearing in honey bee colonies and the resultant variation in queen quality. Insectes Sociaux, 46, 372–377.

Hatjina F, Papaefthimiou C, Charistos L, Dogaroglu T, Bouga M, Emmanouil C and Arnold G, 2013. Sublethal doses of imidacloprid decreased size of hypopharyngeal glands and respiratory rhythm of honeybees in vivo. Apidologie, 44, 467–480.

Hatjina F, Bienkowska M, Charistos L, Chlebo R, Costa C, Drazic MM, Filipi J, Gregorc A, Ivanova EN, Kezic N, Kopernicky J, Kryger P, Lodesani M, Lokar V, Mladenovic M, Panasiuk B, Petrov PP, Rasic S, Skerl MIS, Vejsnaes F and Wilde J, 2014a. A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies. Journal of Apicultural Research, 53, 337–363.

Hatjina F, Costa C, Buechler R, Uzunov A, Drazic M, Filipi J, Charistos L, Ruottinen L, Andonov S, Meixner MD, Bienkowska M, Dariusz G, Panasiuk B, Le Conte Y, Wilde J, Berg S, Bouga M, Dyrba W, Kiprijanovska H, Korpela S, Kryger P, Lodesani M, Pechhacker H, Petrov P and Kezic N, 2014b. Population dynamics of European honey bee genotypes under different environmental conditions. Journal of Apicultural Research, 53, 233–247.

Haubruge E, Nguyen BK, Widart J, Thomé J-P, Fickers P and Depauw E, 2006. Le dépérissement de l'abeille domestique, Apis mellifera L., 1758 (Hymenoptera: Apidae): faits et causes probables. Notes Fauniques de Gembloux, 59, 3–21.

Haxaire J, Bouguet JP and Tamisier JP, 2006. Vespa velutina Lepeletier, 1836, une redoutable nouveauté pour la faune de France (Hym., Vespidae). Bulletin de la Société Entomologique de France, 111, 194.

Haydak MH, 1970. Honey bee nutrition. Annual Review of Entomology, 15, 143–156.

Hendrikx P, Chauzat MP, Debin M, Neumann P, Fries I, Ritter W … and Gregorc A, 2009. Bee mortality and bee surveillance in Europe. EFSA supporting publication 2009;6(9):EN-27, 217 pp. http://www.efsa.europa.eu/en/supporting/pub/27e

Hendrix DL, Wei Y and Leggett JE, 1992. Homopteran honeydew sugar composition is determined by both the insect and plant species. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 101, 23–27.

Henry M, Beguin M, Requier F, Rollin O, Odoux J-F, Aupinel P, Aptel J, Tchamitchian S and Decourtye A, 2012. A common pesticide decreases foraging success and survival in honey bees. Science, 336, 348–350.

Henry M, Cerrutti N, Aupinel P, Decourtye A, Gayrard M, Odoux J-F, Pissard A, Rueger C and Bretagnolle V, 2015. Reconciling laboratory and field assessments of neonicotinoid toxicity to honeybees. Proceedings of the Royal Society B-Biological Sciences, 282, p. 20152110. doi: 10.1098/rspb.2015.2110.

Hepburn HR, 1986. *Honeybees and Wax. An Experimental Natural History*. Springer-Verlag, Berlin.

Hernán MA and Robins JM, 2016. Causal Inference. Chapman and Hall/CRC, Boca Raton, FL, USA, forthcoming.

Higes M, Martín R and Meana A, 2006. *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. Journal of Invertebrate Pathology, 92, 93–95.

Higes M, Martín-Hernández R, Botías C, Bailón EG, González-Porto AV, Barrios L, Del Nozal MJ, Bernal JL, Jiménez JJ and Palencia PG, 2008. How natural infection by *Nosema ceranae* causes honeybee colony collapse. Environmental Microbiology, 10, 2659–2669.
Hladun KR, Parker DR, Tran KD and Trumble JT, 2013. Effects of selenium accumulation on phytotoxicity, herbivory, and pollination ecology in radish (*Raphanus sativus* L.). Environmental Pollution, 172, 70–75.
Hölldobler B and Wilson EO, 2009. *The Superorganism: The Beauty, Elegance, and Strangeness of Insect Societies*. WW Norton & Company, NY, USA.
Hooper T, 2010. *Guide to Bees and Honey*. Northern Bee Books, Hebden Bridge, UK.
Hornitzky MA, 1987. Prevalence of virus infections of honeybees in Eastern Australia. Journal of Apicultural Research, 26, 181–185.
Hornitzky M, 2001. Literature review of chalkbrood – a fungal disease of honeybees. A report for the rural industries research and development corporation. New South Wales Agriculture, AU, Publication 01/150; 13 pp.
Hrassnigg N and Crailsheim K, 1998. Adaptation of hypopharyngeal gland development to the brood status of honeybee (Apis mellifera L.) colonies. Journal of Insect Physiology. 44, 929–939. doi: 10.1016/S0022-1910(98)00058-4
Huang Z-Y and Robinson GE, 1996. Regulation of honey bee division of labor by colony age demography. Behavioral Ecology and Sociobiology, 39, 147–158.
Huang Q, Kryger P, Le Conte Y and Moritz RFA, 2012. Survival and immune response of drones of a Nosemosis tolerant honey bee strain towards *N. ceranae* infections. Journal of Invertebrate Pathology, 109, 297–302.
Human H, Brodschneider R, Dietemann V, Dively G, Ellis JD, Forsgren E, Fries I, Hatjina F, Hu F-L and Jaffé R, 2013. Miscellaneous standard methods for *Apis mellifera* research. Journal of Apicultural Research, 52, 1–53.
Hunt G, 2007. Flight and fight: a comparative view of the neurophysiology and genetics of honey bee defensive behavior. Journal of Insect Physiology, 53, 399–410.
Imdorf A, Bühlmann G, Gerig L, Kilchenmann V and Wille H, 1987. Uberprüfung der Schätzmethode zur Ermittlung der Brutfläche und der Anzahl Arbeiterinnen in freifliegenden Bienenvölkern. Apidologie, 18, 137.
Jackson JT, Tarpy DR and Fahrbach SE, 2011. Histological estimates of ovariole number in honey bee queens, Apis mellifera, reveal lack of correlation with other queen quality measures. Journal of Insect Science, 11, 82.
Jackson CH, Jit M, Sharples LD and De Angelis D, 2015. Calibration of complex models through Bayesian evidence synthesis: a demonstration and tutorial. Medical Decision Making, 35, 148–161.
Jacques A, Laurent M, Ribiere-Chabert M, Saussac M, Bougeard S, Hendrikx P and Chauzat MP, 2016. Statistical analysis on the EPILOBEE dataset: explanatory variables related to honeybee colony mortality in EU during a 2 year survey. EFSA Supporting Publications, 13.
Jalal H, Dowd B, Sainfort F and Kuntz KM, 2013. Linear regression metamodeling as a tool to summarize and present simulation model results. Medical Decision Making, p. 0272989X13492014. doi: 10.1177/0272989X13492014
Johansen CA, 1977. Pesticides and pollinators. Annual Review of Entomology, 22, 177–192.
Johnson BR, 2003. Organization of work in the honeybee: a compromise between division of labour and behavioural flexibility. Proceedings of the Royal Society B-Biological Sciences, 270, 147–152.
Johnson BR, 2010. Division of labor in honeybees: form, function, and proximate mechanisms. Behavioral Ecology and Sociobiology, 64, 305–316.
Johnson JD and Pettis JS, 2014. A survey of imidacloprid levels in water sources potentially frequented by honeybees (Apis mellifera) in the eastern USA. Water, Air, & Soil Pollution, 225, 1–6.
Johnson RM, 2015. Honey bee toxicology. Annual Review of Entomology, 60, 415.
Johnson RM, Ellis MD, Mullin CA and Frazier M, 2010. Pesticides and honey bee toxicity – USA. Apidologie, 41, 312–331.
Johnson RM, Dahlgren L, Siegfried BD and Ellis MD, 2013. Acaricide, fungicide and drug interactions in honey bees (*Apis mellifera*). PLoS ONE, 8, e54092. doi.org/10.1371/journal.pone.0054092
Jones JC, Helliwell P, Beekman M, Maleszka R and Oldroyd BP, 2005. The effects of rearing temperature on developmental stability and learning and memory in the honey bee, Apis mellifera. Journal of Comparative Physiology A, 191, 1121–1129.
Jowell R, Roberts C, Fitzgerald R and Eva G, 2007. *Measuring Attitudes Cross-Nationally – Lessons from the European Social Survey*. Sage, London, UK.
Kahya Y, Gençer HV and Woyke J, 2008. Weight at emergence of honey bee (Apis mellifera caucasica) queens and its effect on live weights at the pre and post mating periods. Journal of Apicultural Research, 47, 118–125.
Kandziora M, Burkhard B and Müller F, 2013. Mapping provisioning ecosystem services at the local scale using data of varying spatial and temporal resolution. Ecosystem Services, 4, 47–59.
Kaur G and Sihag R, 1994. Effects of weather factors on the colony of honeybee (*Apis mellifera* L.). Indian Bee Journal, 56, 158–162.
Keller I, Fluri P and Imdorf A, 2005. Pollen nutrition and colony development in honey bees: part 1. Bee World, 86, 3–10.
Kennedy MC and O'Hagan A, 2001. Bayesian calibration of computer models. Journal of the Royal Statistical Society: Series B (Statistical Methodology), 63, 425–464.

Khallaayoune K, Qualls WA, Revay EE, Allan SA, Arheart KL, Kravchenko VD, Xue R-D, Schlein Y, Beier JC and Müller GC, 2013. Attractive toxic sugar baits: control of mosquitoes with the low-risk attractive ingredient dinotefuran and potential impacts on nontarget organisms in Morocco. Environmental Entomology, 42, 1040–1045. doi: 10.1603/EN13119

Khongphinitbunjong K, de Guzman LI, Burgett MD, Rinderer TE and Chantawannakul P, 2012. Behavioral responses underpinning resistance and susceptibility of honeybees to *Tropilaelaps mercedesae*. Apidologie, 43, 590–599.

Khoury DS, Myerscough MR and Barron AB, 2011. A quantitative model of honey bee colony population dynamics. PLoS ONE, 6, e18491. doi: 10.1371/journal.pone.0018491.

Klee J, Besana AM, Genersch E, Gisder S, Nanetti A, Tam DQ, Chinh TX, Puerta F, Ruz JM, Kryger P, Message D, Hatjina F, Korpela S, Fries I and Paxton RJ, 2007. Widespread dispersal of the microsporidian Nosema ceranae, an emergent pathogen of the western honey bee, *Apis mellifera*. Journal of Invertebrate Pathology, 96, 1–10.

Klein AM, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C and Tscharntke T, 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society of London B: Biological Sciences, 274, 303–313.

Klepper B and Kaufmann MR, 1966. Removal of salt from xylem sap by leaves and stems of guttating plants. Plant Physiology, 41, 1743–1747.

Kluser SU, Neumann P, Chauzat MP and Pettis JS, 2010. *UNEP Emerging Issues: Global Honey Bee Colony Disorder and Other Threats to Insect Pollinators*. United Nations Environment Programme, Nairobi 16pp.

Koeniger N, Koeniger G and Delfinado-Baker M, 1983. Observation on mites of the Asian honeybee species (*Apis cerana*, *Apis dorsata*, *Apis florea*). Apidologie, 14, 197–204.

Koh I, Lonsdorf EV, Williams NM, Brittain C, Isaacs R, Gibbs J and Ricketts TH, 2016. Modeling the status, trends, and impacts of wild bee abundance in the United States. Proceedings of the National Academy of Sciences, 113, 140–145.

Koller D and Friedman N, 2009. *Probabilistic Graphical Models: Principles and Techniques*. MIT Press, Massachusetts, EUA. 1280 pp.

Kottek M, Grieser J, Beck C, Rudolf B and Rubel F, 2006. World map of the Köppen-Geiger climate classification updated. Meteorologische Zeitschrift, 15, 259–263.

Kovac H, Stabentheiner A and Schmaranzer S, 2010. Thermoregulation of water foraging honeybees—balancing of endothermic activity with radiative heat gain and functional requirements. Journal of Insect Physiology, 56, 1834–1845.

Krupke CH, Hunt GJ, Eitzer BD, Andino G and Given K, 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS ONE, 7, e29268. doi.org/10.1371/journal.pone.0029268

Kühnholz S and Seeley TD, 1997. The control of water collection in honey bee colonies. Behavioral Ecology and Sociobiology, 41, 407–422.

Kukielka D and Sánchez-Vizcaíno JM, 2009. One-step real-time quantitative PCR assays for the detection and field study of Sacbrood honeybee and Acute bee paralysis viruses. Journal of Virological Methods, 161, 240–246.

Kuussaari M, Hyvönen T and Härmä O, 2011. Pollinator insects benefit from rotational fallows. Agriculture, Ecosystems and Environment, 143, 28–36.

Lambert O, Veyrand B, Durand S, Marchand P, Le Bizec B, Piroux M, Puyo S, Thorin C, Delbac F and Pouliquen H, 2012. Polycyclic aromatic hydrocarbons: bees, honey and pollen as sentinels for environmental chemical contaminants. Chemosphere, 86, 98–104.

Lambert O, Piroux M, Puyo S, Thorin C, L'Hostis M, Wiest L, Buleté A, Delbac F and Pouliquen H, 2013. Widespread occurrence of chemical residues in beehive matrices from apiaries located in different landscapes of western France. PLoS ONE, 8, e67007.

Landuyt D, Broekx S, D'hondt R, Engelen G, Aertsens J and Goethals PLM, 2013. A review of Bayesian belief networks in ecosystem service modelling. Environmental Modelling and Software, 46, 1–11.

Langridge DF and McGhee RB, 1967. *Crithidia mellificae* an acidophilic trypanosomatid of the honey bee *Apis mellifera*. Journal of Protozoology, 14, 485–487.

Laurent M, Hendrikx P, Ribière-Chabert M and Chauzat MP, 2015. EPILOBEE. A pan-European epidemiological study on honeybee colony losses 2012–2014. EURL, Sophia-Atopolis, France, pp. 1–44.

Le Conte Y, Ellis M and Ritter W, 2010. *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? Apidologie, 41, 353–363.

Ledoux MN, Winston ML, Higo H, Keeling CI, Slessor KN and LeConte Y, 2001. Queen and pheromonal factors influencing comb construction by simulated honey bee (Apis mellifera L.) swarms. Insectes Sociaux, 48, 14–20.

Lee LA, Carslaw KS, Pringle KJ, Mann GW and Spracklen DV, 2011. Emulation of a complex global aerosol model to quantify sensitivity to uncertain parameters. Atmospheric Chemistry and Physics, 11, 12253–12273.

Lee JS, Filatova T, Ligmann-Zielinska A, Hassani-Mahmooei B, Stonedahl F, Lorscheid I and Parker DC, 2015. The complexities of agent-based modeling output analysis. Journal of Artificial Societies and Social Simulation, 18, 4.

Leemon D, 2012. In-Hive Fungal Biocontrol of Small Hive Beetle. RIRDC Publication No. 12/012. RIRDC, Barton, ACT, Australia. p. 69.

Leemon D and McMahon J, 2009. Feasibility Study into In-Hive Fungal Bio-Control of Small Hive Beetle. RIRDC Publication No. 09/090. RIRDC, Barton, ACT, Australia. p. 30.
Legendre P and Legendre LF, 2012. *Numerical Ecology* (Vol 24). Elsevier, Amsterdam.
Li JL, Cornman RS, Evans JD, Pettis JS, Zhao Y, Murphy C, Peng WJ, Wu J, Hamilton M, Boncristiani HFJ, Zhou L, Hammond J and Chen YP, 2014. Systemic spread and propagation of a plant-pathogenic virus in European honeybees, Apis mellifera. MBio, 5, e00898–13. doi: 10.1128/mBio.00898-13
Lindström A, Korpela S and Fries I, 2008. Horizontal transmission of *Paenibacillus larvae* spores between honey bee (*Apis mellifera*) colonies through robbing. Apidologie, 39, 515–522.
Liss KN, Mitchell MG, MacDonald GK, Mahajan SL, Méthot J, Jacob AL, Maguire DY, Metson GS, Ziter C and Dancose K, 2013. Variability in ecosystem service measurement: a pollination service case study. Frontiers in Ecology and the Environment, 11, 414–422.
Liu Y, Yan L, Li Z, Huang WF, Pokhrei S, Liu X and Su S, 2016. Larva-mediated chalkbrood resistance-associated single nucleotide polymorphism markers in the honey bee *Apis mellifera*. Insect Molecular Biology, 25, 239–250.
Lodesani M, Balduzzi D and Galli A, 2004. A study on spermatozoa viability over time in honey bee (Apis mellifera ligustica) queen spermathecae. Journal of Apicultural Research, 43, 27–28.
Lommel SA, Morris TJ and Pinnock DE, 1985. Characterization of nucleic acids associated with Arkansas bee virus. Intervirology, 23, 199–207.
Lonsdorf E, Kremen C, Ricketts TH, Winfree R, Williams NM and Greenleaf SS, 2009. Modelling pollination services across agricultural landscapes. Annals of Botany, 103, 1589–1600.
Louveaux J, Maurizio A and Vorwohl G, 1978. Methods of melissopalynology. Bee World, 59, 139–157.
LUCAS (Land Use/Cover Area Frame Survey), 2012. Technical Reference Document: C-3 Land use and land cover classification (revised). Available online: http://ec.europa.eu/eurostat/documents/205002/208012/LUCAS2012_C3-classification_20131004_0.pdf/a71e46b5-a14b-4c9e-83ed-4f973dc139e0
Main AR, Headley JV, Peru KM, Michel NL, Cessna AJ and Morrissey CA, 2014. Widespread use and frequent detection of neonicotinoid insecticides in wetlands of Canada's Prairie Pothole Region. PLoS ONE, 9, e92821.
Mallet N and Charles L, 2001. Auvergne: etude du cycle biologique annuel (CBA) de l'abeille noire locale en 1999-2000. Mellifica. Available online: http://www.mellifica.be/abeille-noire/ecologie/auvergne.html
Mao W, Schuler MA and Berenbaum MR, 2011. CYP9Q-mediated detoxification of acaricides in the honey bee (Apis mellifera). Proceedings of the National Academy of Sciences, 108, 12657–12662.
Mao W, Schuler MA and Berenbaum MR, 2013. Honey constituents up-regulate detoxification and immunity genes in the western honey bee Apis mellifera. Proceedings of the National Academy of Sciences, 110, 8842–8846.
Maori E, Lavi S, Mozes-Koch R, Gantman Y, Peretz Y, Edelbaum O and Sela I, 2007. Isolation and characterization of Israeli acute paralysis virus, a dicistrovirus affecting honeybees in Israel: evidence for diversity due to intra- and inter-species recombination. Journal of General Virology, 88, 3428–3438.
Mardia KV, Kent JT and Bibby JM, 1979. *Multivariate Analysis*. Academic Press, New York, NY, USA.
Martin SJ, Hogarth A, van Breda J and Perrett J, 1998. A scientific note on Varroa jacobsoni Oudemans and the collapse of Apis mellifera colonies in the United Kingdom. Apidologie (Celle), 29, 369–370.
Martin C, Salvy M, Provost E, Bagnères A-G, Roux M, Crauser D, Clement J-L and Le Conte Y, 2001. Variations in chemical mimicry by the ectoparasitic mite *Varroa jacobsoni* according to the developmental stage of the host honey-bee *Apis mellifera*. Insect Biochemistry and Molecular Biology, 31, 365–379.
Martin SJ, Highfield AC, Brettell L, Villalobos EM, Budge GE, Powell M, Nikaido S and Schroeder DC, 2012. Global honey bee viral landscape altered by a parasitic mite. Science, 336, 1304–1306.
Marzaro M, Vivan L, Targa A, Mazzon L, Mori N, Greatti M, Petrucco Toffolo E, Di Bernardo A, Giorio C and Marton D, 2011. Lethal aerial powdering of honey bees with neonicotinoids from fragments of maize seed coat. Bulletin of Insectology, 64, 119–126.
Matheson A, 1993. World bee health report. Bee World, 74, 176–212.
Mattila HR and Otis GW, 2006. Influence of pollen diet in spring on development of honey bee (Hymenoptera: Apidae) colonies. Journal of Economic Entomology, 99, 604–613.
Mattila HR and Seeley TD, 2014. Extreme polyandry improves a honey bee colony's ability to track dynamic foraging opportunities via greater activity of inspecting bees. Apidologie, 45, 347–363.
Maurizio A, 1985. *Honigtau-honigtauhonig. Waldtracht und Waldhonig in der Imkerei*. Ehrenwirth, Munich. pp. 268–295.
Mayack C and Naug D, 2009. Energetic stress in the honeybee *Apis mellifera* from *Nosema ceranae* infection. Journal of Invertebrate Pathology, 100, 185–188.
Mayack C and Naug D, 2010. Parasitic infection leads to decline in hemolymph sugar levels in honeybee foragers. Journal of Insect Physiology, 56, 1572–1575.
McMullan JB and Brown MJF, 2009. A qualitative model of mortality in honey bee (Apis mellifera) colonies infested with tracheal mites (*Acarapis woodi*). Experimental and Applied Acarology, 47, 225–234.
Meixner MD, Costa C, Kryger P, Hatjina F, Bouga M, Ivanova E and Büchler R, 2010. Conserving diversity and vitality for honey bee breeding. Journal of Apicultural Research, 49, 85–92.
Meixner MD, Pinto MA, Bouga M, Kryger P, Ivanove E and Fuchs S, 2013. Standard methods for characterising subspecies and ecotypes of Apis mellifera. Journal of Apicultural Research, 52, 1–28.

Millennium Ecosystem Assessment, 2005. *Ecosystems and Human Well-Being: Synthesis*. Island Press, Washington, DC, USA 155 pp.
Mingxiao M, Yanna Y, Xiaoli X, Lin Z, Yongfei L and Zhidong L, 2014. Genetic characterization of VP1 gene of seven Sacbrood virus isolated from three provinces in northern China during the years 2008–2012. Virus Research, 176, 78–82.
de Miranda JR, 2008. Diagnostic techniques for virus detection in honey bees. In: Aubert M, Ball B, Fries I, Moritz R, Milani N and Bernardinelli I (eds.). *Virology and the Honey Bee*. European Commission Directorate-General for Research, Brussells, Belgium.
de Miranda JR, Cordoni G and Budge G, 2010. The acute bee paralysis virus–Kashmir bee virus–Israeli acute paralysis virus complex. Journal of Invertebrate Pathology, 103, 30–47.
Moeller FE, 1977. Overwintering of honey bee colonies. Production Research Report-Agricultural Research Service, US Department of Agriculture, USA.
Monceau K, Bonnard O and Thiéry D, 2013. Vespa velutina: a new invasive predator of honeybees in Europe. Journal of Pest Science, doi:10.1007/s10340-013-0537-3
Monceau K, Bonnard O and Thiéry D, 2014. Vespa velutina: a new invasive predator of honeybees in Europe. Journal of Pest Science, 87, 1–16.
Moore AJ, Breed MD and Moor MJ, 1987. The guard honey bee: ontogeny and behavioural variability of workers performing a specialized task. Animal Behaviour, 35, 1159–1167.
Moore J, Jironkin A, Chandler D, Burroughs N, Evans DJ and Ryabov EV, 2011. Recombinants between Deformed wing virus and Varroa destructor virus-1 may prevail in Varroa destructor-infested honeybee colonies. Journal of General Virology, 92, 156–161.
Moritz RF and Fuchs S, 1998. Organization of honeybee colonies: characteristics and consequences of a superorganism concept. Apidologie, 29, 7–22.
Morse RA and Flottum K, 1997. *Honey Bee Pests, Predators and Diseases*, 3rd Edition. A.I. Root, Medina, OH, USA.
Morton D, Rowland C, Wood C, Meek L, Marston C, Smith G, Wadsworth R and Simpson I, 2011. *Final Report for LCM2007 - the new UK land cover map*. Countryside Survey Technical Report No 11/07. NERC/Centre for Ecology & Hydrology, 112 pp. (CEH Project Number: C03259) http://nora.nerc.ac.uk/14854/
Mossadegh MS, 1991. Geographical distribution, levels of infestation and population density of the mite Euvarroa sinhai Delfinado and Baker(Acarina: Mesostigmata) in Apis florea F colonies in Iran. Apidologie, 22, 127–134.
Mouches C, Bove JM, Albisetti J, Clark TB and Tully JG, 1982. A spiroplasma of serogroup-IV causes a May-disease-like disorder of honeybees in southwestern France. Microbial Ecology, 8, 387–399.
Mouches C, Bové JM, Tully JG, Rose DL, McCoy RE, Carle-Junca P, Garnier M and Saillard C, 1983. Spiroplasma apis, a new species from the honey-bee Apis mellifera. In Annales de l'Institut Pasteur/Microbiologie (Vol. 134, No. 3, pp. 383-397). Elsevier Masson.
Mouches C, Bové JM and Albisetti J, 1984. Pathogenicity of Spiroplasma apis and other spiroplasmas for honey-bees in southwestern France. In Annales de l'Institut Pasteur/Microbiologie (Vol. 135, No. 1, pp. 151-155). Elsevier Masson.
Müller HG, Stadtmüller U and Tabnak F, 1997. Spatial smoothing of geographically aggregated data, with application to the construction of incidence maps. Journal of the American Statistical Association, 92, 61–71.
Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R and Pettis JS, 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLoS ONE, 5, e9754.
Mürrle TM, Neumann P, Dames JF, Hepburn HR and Hill MP, 2006. Susceptibility of adult *Aethina tumida* (Coleoptera: Nitidulidae) to entomopathogenic fungi. Journal of Economic Entomology, 99, 1–6.
Muséum National d'Histoire Naturelle (MNHM) and European Environment Agency (EEA), 2014. Terrestrial Habitat Mapping in Europe: An Overview. EEA technical report 1/2014.
Mutinelli F, Montarsi F, Federico G, Granato A, Maroni Ponti A, Grandinetti G, Ferrè N, Franco S, Duquesne V, Rivière M-P, Thiéry R, Henrikx P, Ribière-Chabert M and Chauzat M-P, 2014. Detection of *Aethina tumida* Murray (Coleoptera: Nitidulidae.) in Italy: outbreaks and early reaction measures. Journal of Apicultural Research, 53, 569–575.
Nakano H, Kizaki H and Sakaguchi G, 1994. Multiplication of *Clostridium botulinum* in dead honey-bees and bee pupae, a likely source of heavy contamination of honey. International Journal of Food Microbiology, 23, 247–252.
Nazzi F, Brown SP, Annoscia D, Del Piccolo F, Di Prisco G, Varricchio P, Della Vedova G, Cattonaro F, Caprio E and Pennacchio F, 2012. Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. PLoS Pathogens, 8, e1002735.
Neapolitan RE, 1989. *Probabilistic Reasoning in Expert Systems: Theory and Algorithms*. John Wiley and Sons Inc, New York, NY, USA.
Neumann P and Hoffmann D, 2008. Small hive beetle diagnosis and control in naturally infested honeybee colonies using bottom board traps and CheckMite+ strips. Journal of Pest Science, 81, 43–48.
Nevas M, Hielm S, Lindstrom M, Koivulehto K, Horn H and Korkeala H, 2002. High prevalence of Clostridium botulinum types A and B in honey samples detected by polymerase chain reaction. International Journal of Food Microbiology, 72, 45–52.

Nielsen SL, Nicolaisen M and Kryger P, 2008. Incidence of acute bee paralysis virus, black queen cell virus, chronic bee paralysis virus, deformed wing virus, Kashmir bee virus and sacbrood virus in honey bees (Apis mellifera) in Denmark. Apidologie, 39, 310–314.
Nolan MP and Delaplane KS, 2016. Distance between honey bee *Apis mellifera* colonies regulates populations of Varroa destructor at a landscape scale. Apidologie, 1–9. doi: 10.1007/s13592-016-0443-9
Nordstrom S and Fries I, 1995. A comparison of media and cultural conditions for identification of *Bacillus* larvae in honey. Journal of Apicultural Research, 34, 97–103.
Oakley JE and Youngman BD, 2015. Calibration of stochastic computer simulators using likelihood emulation. Technometrics. Available online: http://dx.doi.org/10.1080/00401706.2015.1125391
Odoux J-F, Aupinel P, Gateff S, Requier F, Henry M and Bretagnolle V, 2014. ECOBEE: a tool for long-term honey bee colony monitoring at the landscape scale in West European intensive agroecosystems. Journal of Apicultural Research, 53, 57–66.
Odoux J-F, Feuillet D, Aupinel P, Loublier Y, Tasei J-N and Mateescu C, 2012. Territorial biodiversity and consequences on physico-chemical characteristics of pollen collected by honey bee colonies. Apidologie, 43, 561–575.
Odoux J-F, Aupinel P, Gateff S, Requier F, Henry M and Bretagnolle V, 2014. ECOBEE: a tool for long-term honey bee colony monitoring at the landscape scale in West European intensive agroecosystems. Journal of Apicultural Research, 53, 57–66.
Oldroyd BP and Ratnieks FLW, 2000. Evolution of worker sterility in honey-bees (*Apis mellifera*): how anarchistic workers evade policing by laying eggs that have low removal rates. Behavioral Ecology and Sociobiology, 47, 268–273.
Olsson O and Bolin A, 2014. A model for habitat selection and species distribution derived from central place foraging theory. Oecologia, 175, 537–548. Available online: http://www.ncbi.nlm.nih.gov/pubmed/24696358
Ongus JR, Peters D, Bonmatin JM, Bengsch E, Vlak JM and van Oers MM, 2004. Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite Varroa destructor. Journal of General Virology, 85, 3747–3755.
Orantes-Bermejo FJ, Pajuelo AG, Megías MM and Fernández-Píñar CT, 2010. Pesticide residues in beeswax and bee bread samples collected from honey bee colonies (*Apis mellifera* L.) in Spain. Possible implications for bee losses. Journal of Apicultural Research, 49, 243–250.
Otis GW and Scott-Dupree CD, 1992. Effects of *Acarapis woodi* on overwintered colonies of honey bees (Hymenoptera: Apidae) in New York. Journal of Economical Entomology, 85, 40–46.
Özgör E, Güzerin E and Keskin N, 2015. Determination and comparison of *Nosema apis* and *Nosema ceranae* in terms of geographic and climatic factors. Hacettepe Journal of Biology and Chemistry, 2015, 43, 9–15.
Page RE and Peng CY-S, 2001. Aging and development in social insects with emphasis on the honey bee, *Apis mellifera* L. Experimental Gerontology, 36, 695–711.
Page RE, Waddington KD, Hunt GJ and Fondrk MK, 1995. Genetic determinants of honey bee foraging behaviour. Animal Behaviour, 50, 1617–1625.
Palma MS, 1992. Composition of freshly harvested Brazilian royal jelly: identification of carbohydrates from the sugar fraction. Journal of Apicultural Research, 31, 42–44.
Palmeri V, Scirtò G, Malacrinò A, Laudani F and Campolo O, 2015. A scientific note on a new pest for European honeybees: first report of small hive beetle *Aethina tumida* (Coleoptera: Nitidulidae) in Italy. Apidologie, 46, 527–529.
Pankiw T and Page RE, 2001. Genotype and colony environment affect honeybee (Apis mellifera L.) development and foraging behavior. Behavioral Ecology and Sociobiology, 51, 87–94.
Panseri S, Catalano A, Giorgi A, Arioli F, Procopio A, Britti D and Chiesa LM, 2014. Occurrence of pesticide residues in Italian honey from different areas in relation to its potential contamination sources. Food Control, 38, 150–156.
Parajka J and Blöschl G, 2008. The value of MODIS snow cover data in validating and calibrating conceptual hydrologic models. Journal of Hydrology, 358, 240–258.
Parajka J, Kohnová S, Merz R, Szolgay J, Hlavčová K and Blöschl G, 2009. Comparative analysis of the seasonality of hydrological characteristics in Slovakia and Austria/Analyse comparative de la saisonnalité de caractéristiques hydrologiques en Slovaquie et en Autriche. Hydrological Sciences Journal, 54, 456–473.
Parker BJ, Barribeau SM, Laughton AM, de Roode JC and Gerardo NM, 2011. Non-immunological defense in an evolutionary framework. Trends in Ecology & Evolution, 26, 242–248.
Paxton RJ, Klee J, Korpela S and Fries I, 2007. *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. Apidologie, 38, 558–565.
Pearl J, 1985. *Bayesian Networks: A Model of Self-Activated Memory for Evidential Reasoning*. Computer Science Department, University of California, Los Angeles, CA, USA.
Pearl J, 1995. Probabilistic reasoning in intelligent systems: networks of plausible inference. Synthese-Dordrecht, 104, 161.
Pearl J, 2009. Causality. Cambridge University Press. 464 pp.
Pérez-Sato JA, Châline N, Martin SJ, Hughes WOH and Ratnieks FL, 2009. Multi-level selection for hygienic behaviour in honeybees. Heredity, 102, 609–615.

Pernal SF, Albright RL and Melathopoulos AP, 2008. Evaluation of the shaking technique for the economic management of American foulbrood disease of honey bees (Hymenoptera: Apidae). Journal of Economic Entomology, 101, 1095–1104.
Pernal SF and Melathopoulos AP, 2006. Monitoring for American foulbrood spores from honey and bee samples in Canada. Apiacta, 41, 99–109.
Perry CJ, Søvik E, Myerscough MR and Barron AB, 2015. Rapid behavioral maturation accelerates failure of stressed honey bee colonies. Proceedings of the National Academy of Sciences, 112, 3427–3432.
Petanidou T and Vokou D, 1990. Pollination and pollen energetics in Mediterranean ecosystems. American Journal of Botany, 77, 986–992.
Pettis J, Collins A, Wilbanks R and Feldlaufer MF, 2004. Effects of coumaphos on queen rearing in the honey bee, *Apis mellifera*. Apidologie, 35, 605–610.
Pettis JS, vanEngelsdorp D, Johnson J and Dively G, 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*. Naturwissenschaften, 99, 153–158.
Pettis JS, Rice N, Joselow K and Chaimanee V, 2016. Colony failure linked to low sperm viability in honey bee (Apis mellifera) queens and an exploration of potential causative factors. PLoS ONE, 112, e0147220.
Pilling E, Campbell P, Coulson M, Ruddle N and Tornier I, 2013. A four-year field program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam. PLoS ONE, 8, e77193. doi: 10.1371/journal.pone.0077193
Pohorecka K, Zdańska D, Bober A and Skubida M, 2011. First detection of Israeli acute paralysis virus (IAPV) in Poland and phylogenetic analysis of the isolates. Journal of Apicultural Science, 55, 149–59.
Polce C, Termansen M, Aguirre-Gutiérrez J, Boatman ND, Budge GE, Crowe A, Garratt MP, Pietravalle S, Potts SG, Ramirez JA and Somerwill KE, 2013. Species distribution models for crop pollination: a modelling framework applied to Great Britain. PLoS ONE, 8, e76308. doi.org/10.1371/journal.pone.0076308
Porrini C, Mutinelli F, Bortolotti L, Granato A, Laurenson L, Roberts K, Gallina A, Silvester N, Medrzycki P, Renzi T, Sgolastra F and Lodesani M, 2016. The status of honey bee health in Italy: results from the nationwide bee monitoring network. PLoS ONE, 115, e0155411.
Potts SG, Vulliamy B, Dafni A, Ne'eman G, O'Toole C, Roberts S and Willmer P, 2003a. Response of plant-pollinator communities to fire: changes in diversity, abundance and floral reward structure. Oikos, 101, 103–112.
Potts SG, Vulliamy B, Dafni A, Ne'eman G and Willmer P, 2003b. Linking bees and flowers: how do floral communities structure pollinator communities? Ecology, 84, 2628–2642.
Potts SG, Petanidou T, Roberts S, O'Toole C, Hulbert A and Willmer P, 2006. Plant–pollinator biodiversity and pollination services in a complex Mediterranean landscape. Biological Conservation, 129, 519–529.
Potts SG, Roberts SP, Dean R, Marris G, Brown MA, Jones R, Neumann P and Settele J, 2010. Declines of managed honey bees and beekeepers in Europe. Journal of Apicultural Research, 49, 15–22.
Pratt SC, 1998. Decentralized control of drone comb construction in honey bee colonies. Behavioral Ecology and Sociobiology, 42, 193–205.
Presoto AEF, Rios MD and Almeida-Muradian LBD, 2004. Simultaneous high performance liquid chromatographic analysis of vitamins B1, B2 and B6 in royal jelly. Journal of the Brazilian Chemical Society, 15, 136–139.
Railsback SF and Grimm V, 2011. *Agent-Based and Individual-Based Modeling: A Practical Introduction*. Princeton University Press, Princeton, NJ, USA.
du Rand EE, Smit S, Beukes M, Apostolides Z, Pirk CWW and Nicolson SW, 2015. Detoxification mechanisms of honey bees (Apis mellifera) resulting in tolerance of dietary nicotine. Scientific Reports, 5, 11779. doi: 10.1038/srep11779
Ravoet J, Maharramov J, Meeus I, De Smet L, Wenseleers T, Smagghe G and De Graaf D, 2013. Comprehensive bee pathogen screening in Belgium reveals *Crithidia mellificae* as a new contributory factor to winter mortality. PLoS ONE, 8, e72443.
Requier F, Odoux J-F, Tamic T, Moreau N, Henry M, Decourtye A and Bretagnolle V, 2015. Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. Ecological Applications, 25, 881–890.
Ribière M, Triboulot C, Mathieu L, Aurières C, Faucon J-P and Pépin M, 2002. Molecular diagnosis of chronic bee paralysis virus infection. Apidologie, 33, 339–352.
Ribière M, Ball B and Aubert M, 2008. Natural history and geographical distribution of honey bee In: viruses. Virology and the Honey bee: Office for Official Publications of the European Communities Brussels. 15–84.
Ribière M, Olivier V and Blanchard P, 2010. Chronic bee paralysis: a disease and a virus like no other? Journal of Invertebrate Pathology, 103, 120–131.
Richards CS, Hill MP and Dames JF, 2005. The susceptibility of small hive beetle (*Aethina tumida* Murray) pupae to *Aspergillus niger* (van Tieghem) and *A. flavus* (Link: Grey). American Bee Journal, 145, 748–751.
Riddell Pearce FC, Couvillon MJ and Ratnieks FL, 2013. Hive relocation does not adversely affect honey bee (Hymenoptera: Apidae) foraging. Psyche: A Journal of Entomology, 693856. doi:10.1155/2013/693856
Riley JR, Greggers U, Smith AD, Reynolds DR and Menzel R, 2005. The flight paths of honeybees recruited by the waggle dance. Nature, 435, 205–207.
Ritter W, 2003. Early detection of American foulbrood by honey and wax analysis. Apiacta, 38, 125–130.

Rivière M-P, Ribière M and Chauzat M-P, 2013. Recent molecular biology methods for foulbrood and nosemosis diagnosis. Revue Scientifique et Technique, 32, 885–892.
Robinson GE and Huang ZY, 1998. Colony integration in honey bees: genetic, endocrine and social control of division of labor. Apidologie, 29, 159–170.
Robinson GE, Page RE, Strambi C and Strambi A, 1992. Colony integration in honey bees: mechanisms of behavioral reversion. Ethology, 904, 336–348.
Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C and Pounds JA, 2003. Fingerprints of global warming on wild animals and plants. Nature, 421, 57–60. doi: 10.1038/nature01333.
Rosenkranz P, Aumeier P and Ziegelmann B, 2010. Biology and control of *Varroa destructor*. Journal of Invertebrate Pathology, 103, S96–S119.
Roubik DW and Buchmann SL, 1984. Nectar selection by *Melipona* and *Apis mellifera* (Hymenoptera: Apidae) and the ecology of nectar intake by bee colonies in a tropical forest. Oecologia, 61, 1–10.
Roulston TH and Cane JH, 2000. Pollen nutritional content and digestibility for animals. Plant Systematics and Evolution, 222, 187–209.
Ruiz-González MX and Brown MJF, 2006. Honey bee and bumblebee trypanosomatids: specificity and potential for transmission. Ecological Entomology, 31, 616–622.
Rueppell O, Bachelier C, Fondrk MK and Page RE, 2007. Regulation of life history determines lifespan of worker honey bees (Apis mellifera L.). Experimental Gerontology, 42, 1020–1032. doi: 10.1016/j.exger.2007.06.002.
Runckel C, Flenniken ML, Engel JC, Ruby JG, Ganem D, Andino R and DeRisi JL, 2011. Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, *Nosema*, and *Crithidia*. PLoS ONE, 6, e20656.
Runckel C, DeRisi J and Flenniken ML, 2014. A draft genome of the honey bee trypanosomatid parasite *Crithidia mellificae*. PLoS ONE, 9, e95057.
Safavian RS and Landgreb D, 1991. A survey of decision tree classifier methodology. IEEE Transactions on Systems, Man, and Cybernetics, 21, 660–674.
Salazar-Olivo L and Paz-González V, 2005. Screening of biological activities present in honeybee (*Apis mellifera*) royal jelly. Toxicology In Vitro, 19, 645–651.
Sammataro D, Gerson U and Needham G, 2000. Parasitic mites of honey bees: life history, implications and impact. Annual Review of Entomology, 45, 519–548.
Sanchez-Bayo F and Goka K, 2014. Pesticide residues and bees – a risk assessment. PLoS ONE, 9, e94482. doi.org/10.1371/journal.pone.0094482
Santillán-Galicia MT, Ball BV, Clark SJ and Alderson PG, 2010. Transmission of deformed wing virus and slow paralysis virus to adult bees (Apis mellifera L.) by Varroa destructor. Journal of Apicultural Research, 49, 141–148.
Scheiner R, Abramson CI, Brodschneider R, Crailsheim K, Farina WM, Fuchs S, Grünewald B, Hahshold S, Karrer M and Koeniger G, 2013. Standard methods for behavioural studies of *Apis mellifera*. Journal of Apicultural Research, 52, 1–58.
Schmickl T and Crailsheim K, 2001. Cannibalism and early capping: strategy of honeybee colonies in times of experimental pollen shortages. Journal of Comparative Physiology A, 187, 541–547.
Schmickl T and Crailsheim K, 2002. How honeybees (Apis mellifera L.) change their broodcare behaviour in response to non-foraging conditions and poor pollen conditions. Behavioral Ecology and Sociobiology, 515, 415–425.
Schmidt JO, 1984. Feeding preferences of *Apis mellifera* L. (Hymenoptera, Apidae) – individual versus mixed pollen species. Journal of the Kansas Entomological Society, 57, 323–327.
Schmidt JO, Thoenes SC and Levin MD, 1987. Survival of honey nees, *Apis mellifera* (Hymenoptera: Apidae), fed various pollen sources. Annals of the Entomological Society of America, 80, 176–183. Available online: http://aesa.oxfordjournals.org/aesa/80/2/176.full.pdf
Schmidt LS, Schmidt JO, Rao H, Wang W and Xu L, 1995. Feeding preference and survival of young worker honey bees (Hymenoptera: Apidae) fed rape, sesame, and sunflower pollen. Journal of Economic Entomology, 88, 1591–1595.
Schmitzova J, Klaudiny J, Albert Š, Schröder W, Schreckengost W, Hanes J, Judova J and Šimúth J, 1998. A family of major royal jelly proteins of the honeybee *Apis mellifera* L. Cellular and Molecular Life Sciences CMLS, 54, 1020–1030.
Schneider CW, Tautz J, Grünewald B and Fuchs S, 2012. RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. PLoS ONE, 7, e30023.
Schroeder DC and Martin SJ, 2012. Deformed wing virus: the main suspect in unexplained honeybee deaths worldwide. Virulence, 3, 589–591.
Schulp C, Lautenbach S and Verburg P, 2014a. Quantifying and mapping ecosystem services: demand and supply of pollination in the European Union. Ecological Indicators, 36, 131–141.
Schulp CJE, Thuiller W and Verburg PH, 2014b. Wild food in Europe: a synthesis of knowledge and data of terrestrial wild food as an ecosystem service. Ecological Economics, 105, 292–305.

Schwarz RS, Bauchan GR, Murphy CA, Ravoet J, de Graaf DC and Evans JD, 2015. Characterization of two species of Trypanosomatidae from the honey bee *Apis mellifera*: *Crithidia mellificae* Langridge and McGhee, 1967 and *Lotmaria passim* n. gen., n. sp. Journal of Eukaryotic Microbiology, 62(5), 567–583. doi: 10.1111/jeu.12209

Scofield HN and Mattila HR, 2015. Honey bee workers that are pollen stressed as larvae become poor foragers and waggle dancers as adults. PLoS ONE, 104.

Seeley TD, 1978. Life history strategy of the honey bee, Apis mellifera. Oecologia, 32, 109–118.

Seeley TD, 1982. Adaptive significance of the age polyethism schedule in honeybee colonies. Behavioral Ecology and Sociobiology, 11, 287–293.

Seeley TD, 1985. *Honey Bee Ecology. Monographs in Behavior and Ecology*. Princeton University Press, Princeton, NJ, USA.

Seeley, TD, 1989. Social foraging in honey bees: how nectar foragers assess their colony's nutritional status. Behavioral Ecology and Sociobiology, 24, 181–199. doi: 10.1007/BF00292101.

Seeley TD, 1995. *The wisdom of the hive: the social physiology of honey bee*. Harvard University, Cambridge, USA. 318 pp.

Seeley TD, 1997. Honey bee colonies are group-level adaptive units. The American Naturalist, 150, S22–S41.

Seeley TD and Smith ML, 2015. Crowding honeybee colonies in apiaries can increase their vulnerability to the deadly ectoparasite *Varroa destructor*. Apidologie, 46, 716–727.

Seeley TD and Visscher P, 1985. Survival of honeybees in cold climates: the critical timing of colony growth and reproduction. Ecological Entomology, 10, 81–88.

Shaffer JP, 1995. Multiple hypothesis testing. Annual Review of Psychology, 46, 561–584.

Shipley B, 2000. *Cause and Correlation in Biology*. Cambridge University Press, Cambridge, UK. p. 317.

Shoreit MN and Bagy MMK, 1995. Mycoflora associated with stonebrood disease in honeybee colonies in Egypt. Microbiological Research, 150, 207–2011. doi: 10.1016/S0944-5013(11)80058-3.

Siede R, König M, Büchler R, Failing K and Thiel HJ, 2008. A real-time PCR based survey on acute bee paralysis virus in German bee colonies. Apidologie, 39, 650–661.

Simon-Delso N, San MG, Bruneau E, Minsart L-A, Mouret C and Hautier L, 2014. Honeybee colony disorder in crop areas: the role of pesticides and viruses. PLoS ONE, 9.

Simone M, Evans JD and Spivak M, 2009. Resin collection and social immunity in honey bees. Evolution, 63, 3016–3022. doi:10.1111/j.1558-5646.2009.00772.x

Simone-Finstrom M and Spivak M, 2010. Propolis and bee health: the natural history and significance of resin use by honey bees. Apidologie, 41, 295–311. doi:10.1051/apido/2010016

Škerl MI and Gregorc A, 2010. Heat shock proteins and cell death in situ localisation in hypopharyngeal glands of honeybee (Apis mellifera carnica) workers after imidacloprid or coumaphos treatment. Apidologie, 41, 73–86. doi: 10.1051/apido/2009051.

Slessor KN, Winston ML and Le Conte Y, 2005. Pheromone communication in the honeybee (*Apis mellifera* L.). Journal of Chemical Ecology, 31, 2731–2745.

Smart M, Pettis J, Rice N, Browning Z and Spivak M, 2015. Linking measures of colony and individual honey bee health to survival among apiaries exposed to varying agricultural land use. PLoS ONE, 113, e0152685. doi:10.1371/journal.pone.0152685

Smirle MJ and Winston ML, 1988. Detoxifying enzyme activity in worker honey bees: an adaptation for foraging in contaminated ecosystems. Canadian Journal of Zoology, 66, 1938–1942.

Smith KM, Loh EH, Rostal MK, Zambrana-Torrelio CM, Mendiola L and Daszak P, 2013. Pathogens, pests, and economics: drivers of honey bee colony declines and losses. EcoHealth, 10, 434–445.

Snijder TAB and Bosker RJ, 2012. *Multilevel analysis: an introduction to basic and advanced multilevel modeling*. 2nd Edition, Sage Publishers, London, 368 pp, ISBN 9781849202008

Soares S, Amaral JS, Oliveira MBPP and Mafra I, 2015. Improving DNA isolation from honey for the botanical origin identification. Food Control, 48, 130–136.

Soliman Kamel JS and Sheppard W, 2003. A scientific note on hygienic behavior in *Apis mellifera lamarckii* and *A. m. carnica* in Egypt. Apidologie, 34, 189–190.

Somerville DC and Nicol HI, 2006. Crude protein and amino acid composition of honey bee-collected pollen pellets from south-east Australia and a note on laboratory disparity. Australian Journal of Experimental Agriculture, 46, 141–149.

Southwick E and Moritz R, 1987. Effects of meteorological factors on defensive behaviour of honey bees. International Journal of Biometeorology, 31, 259–265.

Spivak M, 1996. Honey bee hygienic behavior and defense against *Varroa jacobsoni*. Apidologie, 27, 245–260.

Spivak M and Downey DL, 1998. Field assays for hygienic behavior in honey bees (Hymenoptera: Apidae). Journal of Economic Entomology, 91, 64–70.

Spivak M and Gilliam M, 1993. Facultative expression of hygienic behavior of honey-bees in relation to disease resistance. Journal of Apicultural Research, 32, 147–157.

Spivak M and Gilliam M, 1998. Hygienic behaviour of honey bees and its application for control of brood diseases and Varroa: Part II. Studies on hygienic behaviour since the Rothenbuhler era. Bee World, 79, 169–186.

Spoolder H, De Rose G, Hörning B, Waiblinger S and Wemelsfelder F, 2003. Integrating parameters to assess on-farm welfare. Animal Welfare, 12, 529–534.

Stärk KDC, Regula G, Hernandez J, Knopf L, Fuchs K, Morris RS and Davies P, 2006. Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health: review of current approaches. BMC Health Services Research, 6, 20.
Stanley RG and Linskens HF, 1974. *Pollen. Biology Biochemistry Management*. pp. 154–163. DOI: 10.1007/978-3-642-65905-8. ISBN: 978-3-642-65907-2.
Steffan-Dewenter I and Kuhn A, 2003. Honeybee foraging in differentially structured landscapes. Proceedings of the Royal Society of London B: Biological Sciences, 270, 569–575.
Steinmann N, Corona M, Neumann P and Dainat B, 2015. Overwintering is associated with reduced expression of immune genes and higher susceptibility to virus infection in honey bees. PLoS ONE, 10. doi.org/10.1371/journal.pone.0129956
Stocker A, Schramel P, Kettrup A and Bengsch E, 2005. Trace and mineral elements in royal jelly and homeostatic effects. Journal of Trace Elements in Medicine and Biology, 19, 183–189.
Strachecka A, Borsuk G, Olszewski K and Paleolog J, 2015. A new detection method for a newly revealed mechanism of pyrethroid resistance development in *Varroa destructor*. Parasitology Research, 114, 3999–4004.
Strauss U, Dietemann V, Human H, Crewe RM and Pirk CWW, 2016. Resistance rather than tolerance explains survival of savannah honeybees (*Apis mellifera scutellata*) to infestation by the parasitic mite *Varroa destructor*. Parasitology, 143, 374–387.
Streit S, Bock F, Pirk CWW and Tautz J, 2003. Automatic life-long monitoring of individual insect behaviour now possible. Zoology, 106, 169–171.
Struye MH, Mortier HJ, Arnold G, Miniggio C and Borneck R, 1994. Microprocessor-controlled monitoring of honeybee flight activity at the hive entrance. Apidologie, 25, 384–395. Available online: http://dx.doi.org/10.1051/apido:19940405
Sullivan JP, Jassim O, Fahrbach SE and Robinson GE, 2000. Juvenile hormone paces behavioral development in the adult worker honey bee. Hormones and Behavior, 371, 1–14.
Tarpy DR, Hatch S and Fletcher DJ, 2000. The influence of queen age and quality during queen replacement in honeybee colonies. Animal Behaviour, 591, 97–101.
Tarpy DR, vanEngelsdorp D and Pettis JS, 2013. Genetic diversity affects colony survivorship in commercial honey bee colonies. Naturwissenschaften, 100, 723–728.
Tenenhaus M, Esposito Vinzi V, Chatelin YM and Lauro C, 2005. PLS path modelling. Computational Statistics and Data Analysis, 48, 159–205.
Tentcheva D, Gauthier L, Jouve S, Canabady-Rochelle L, Dainat B, Cousserans F, Colin M, Ball B and Bergoin M, 2004. Polymerase Chain Reaction detection of deformed wing virus (DWV) in Apis mellifera and Varroa destructor. Apidologie, 35, 431–439.
The Economics of Ecosystems and Biodiversity (TEEB), 2010. Chapter 1: Integrating the ecological and economic dimensions in biodiversity and ecosystem service valuation. In: Kumar P (ed.) *The Ecological and Economic Foundations*. Earthscan, London, UK. Available online: http://www.teebweb.org/wp-content/uploads/2013/04/D0-Chapter-1-Integrating-the-ecological-and-economic-dimensions-in-biodiversity-and-ecosystem-service-valuation.pdf
The Land Use Database of the Netherlands (LGN7), 2013. Available online at http://www.wageningenur.nl/en/Expertise-Services/Research-Institutes/alterra/Facilities-Products/Land-use-database-of-the-Netherlands.htm
Tian B, Fadhil NH, Powell JE, Kwong WK and Moran NA, 2012. Long-term exposure to antibiotics has caused accumulation of resistance determinants in the gut microbiota of honeybees. MBio, 3, e00377–00312.
Toma B, Dufour B, Sanaa M, Benet JJ, Moutou F, Louza A and Ellis P (eds), 1999. Applied Veterinary Epidemiology and the Control of Disease in Populations. Association pour l'Etude de l'Epidémiologie des Maladies Animales (AEEMA). 536 pp. ISBN 92-9044-487-8.
Tomkies V, Flint J, Johnson G, Ruth W, Wilkins S, Danks C, Watkins M, Cuthbertson G, Carpana E, Marris G, Budge G and Brown M, 2009. Development and validation of a novel field test kit for European foulbrood. Apidologie, 40, 63–72.
Toplak I, Jamnikar Ciglenečki U, Aronstein K and Gregorc A, 2013. Chronic bee paralysis virus and *Nosema ceranae* experimental co-infection of winter honey bee workers (*Apis mellifera* L.). Viruses, 5, 2282–2297.
Trumbo ST, Huang Z-Y and Robinson GE, 1997. Division of labor between undertaker specialists and other middle-aged workers in honey bee colonies. Behavioral Ecology and Sociobiology, 41, 151–163.
United States Environmental Protection Agency (US EPA), 2010. Climate Change Indicators in the United States. Updated June 2015. Available online: https://www3.epa.gov/climatechange/pdfs/print_snow-cover-2015.pdf
Vaissière B, Freitas BM and Gemmill-Herren B, 2011. Protocol to detect and assess pollination deficits in crops: a handbook for its use. Food and agriculture organization of the United Nations, Rome, Italy. Available online: http://www.fao.org/docrep/013/i1929e/i1929e00.htm
Vallon J, Cerrutti N and Jourdan P, 2008. Suivi de colonies d'abeilles lors de la miellée de lavandin sur le plateau de Valensole. Protocol et resultats obtenus en 2007. Bulletin Technique Apicole, 35, 71–80.
Van der Steen JJM, 2015. The foraging honey bee. British Beekeepers Association News, February, 43–46.
van der Steen JS and Brodschneider R, 2014. Public Participation In Bee Science: CSI Pollen. Bee World, 91, 25–27.

Van der Zee R, Pisa L, Andonov S, Brodschneider R, Charrière J-D, Chlebo R, Coffey MF, Crailsheim K, Dahle B and Gajda A, 2012. Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008–9 and 2009–10. Journal of Apicultural Research, 51, 100–114.

Van der Zee R, Gray A, Holzmann C, Pisa L, Brodschneider R, Chlebo R, Coffey MF, Kence A, Kristiansen P and Mutinelli F, 2013. Standard survey methods for estimating colony losses and explanatory risk factors in *Apis mellifera*. Journal of Apicultural Research, 52, 1–36.

Van der Zee R, Gray A, Pisa L and De Rijk T, 2015. An observational study of honey bee colony winter losses and their association with *Varroa destructor*, neonicotinoids and other risk factors. PLoS ONE, 10, e0131611. doi:10.1371/journal.pone.0131611

Varis AL, Ball BV and Allen M, 1992. The incidence of pathogens in honey bee (Apis mellifera L) colonies in Finland and Great Britain. Apidologie, 23, 133–137.

Vásquez A and Olofsson TC, 2009. The lactic acid bacteria involved in the production of bee pollen and bee bread. Journal of Apicultural Research, 48, 189–195.

Verdes PF, 2005. Assessing causality from multivariate time series. Physical Review E, 722, 026222.

Villa JD and Rinderer T, 2008. Inheritance of resistance to *Acarapis woodi* (Acari: Tarsonemidae) in crosses between selected resistant Russian and selected susceptible US honey bees (Hymenoptera: Apidae). Journal of Economic Entomology, 101, 1756–1759.

Villemant C, Haxaire J and Streito JC, 2006. Premier bilan de l'invasion de Vespa velutina Lepeletier en France (Hymenoptera, Vespidae). Bulletin de la Société Entomologique de France, 1114, 535–538.

Villemant C, Barbet-Massin M, Perrard A, Muller F, Gargominy O, Jiguet F and Rome Q, 2011. Predicting the invasion risk by the alien bee-hawking Yellow-legged hornet Vespa velutina nigrithorax across Europe and other continents with niche models. Biological Conservation, 1449, 2142–2150.

Visscher PK, 1983. The honey bee way of death: necrophoric behaviour in Apis mellifera colonies. Animal Behaviour, 314, 1070–1076.

Visscher PK and Seeley TD, 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. Ecology, 63, 1790–1801.

Von der Ohe W, Persano Oddo L, Piana M, Morlot M and Martin P, 2004. Harmonized methods of melissopalynology. Apidologie, 35, S18–S25.

Von Frisch K, 1967. *The Dance Language and Orientation of Bees*. Harvard University Press, Cambridge, MA, USA.

Wackernagel H, 2003. *Multivariate Geostatistics*, 3rd Edition. Springer-Verlag, Berlin 387 pp.

Waite R, Brown M and Thompson H, 2003. Hygienic behaviour in honey bees in the UK: a preliminary study. Bee World, 84, 19–26.

Wang H, Xie J, Shreeve TG, Ma J, Pallett DW, King LA and Possee RD, 2013. Sequence recombination and conservation of Varroa destructor virus-1 and deformed wing virus in field collected honey bees (Apis mellifera). PLoS ONE, 8, e74508.

Wario F, Wild B, Couvillon MJ, Rojas R and Landgraf T, 2015. Automatic methods for long-term tracking and the detection and decoding of communication dances in honeybees. Frontiers in Ecology and Evolution, 3, 103. doi: 10.3389/fevo.2015.00103

White JW Jr, 1975. Physical characteristics of honey. In: Crane E (ed). *Honey: A Comprehensive Survey*. Heinemann, London, UK.

Wiest L, Buleté A, Giroud B, Fratta C, Amic S, Lambert O, Pouliquen H and Arnaudguilhem C, 2011. Multi-residue analysis of 80 environmental contaminants in honeys, honeybees and pollens by one extraction procedure followed by liquid and gas chromatography coupled with mass spectrometric detection. Journal of Chromatography A, 1218, 5743–5756.

Wilkins S, Brown MA and Cuthbertson AGS, 2007. Perspective: the incidence of honey bee pests and diseases in England and Wales. Pest Management Science, 63, 1062–1068.

Williams DL, 2000. A veterinary approach to the European honey bee (Apis mellifera). The Veterinary Journal, 1601, 61–73.

Williams GR, Troxler A, Retschnig G, Roth K, Yañez O, Shutler D, Neumann P and Gauthier L 2015. Neonicotinoid pesticides severely affect honey bee queens. Scientific Reports, 5 doi: 10.1038/srep14621

Wilson EE, Sidhu CS, LeVan KE and Holway DA, 2010. Pollen foraging behaviour of solitary Hawaiian bees revealed through molecular pollen analysis. Molecular Ecology, 19, 4823–4829.

Winfree R, Aguilar R, Vázquez DP, LeBuhn G and Aizen MA, 2009. A meta-analysis of bees' responses to anthropogenic disturbance. Ecology, 90, 2068–2076. Available online: http://dx.doi.org/10.1890/08-1245.1

Winfree R, Gross BJ and Kremen C, 2011. Valuing pollination services to agriculture. Ecological Economics, 71, 80–88.

Winston ML, 1991. *The biology of the honey bee*. Harvard University Press, Massachusetts, EUA 283 pp.

Wold H, 1982. Soft modeling: the basic design and some extensions. In: Joreskog KG and Wold H (eds.). *Systems Under Indirect Observations: Causality, Structure, Prediction, Part 2*. Academic Press, North Holland, Amsterdam, pp. 1–54.

Wolf S, McMahon DP, Lim KS, Pull CD, Clark SJ, Paxton RJ and Osborne JL, 2014. So near and yet so far: harmonic radar reveals reduced homing ability of Nosema infected honeybees. PLoS ONE, 9, e103989.

World Meteorological Organization (WMO), 2008. Guide to Meteorological Instruments and Methods of Observation from the World Meteorological Organization WMO Guidance. Available online: https://www.wmo.int/pages/prog/gcos/documents/gruanmanuals/CIMO/CIMO_Guide-7th_Edition-2008.pdf
World Organisation for Animal Health, 2013. Small hive beetle infestation. OIE Terrestrial Manual, Chapter 2.2.5. OIE, Paris.
World Organisation for Animal Health (OIE), 2008a. European foulbrood of honey bees. OIE Terrestrial Manual, Chapter 2.2.3. OIE, Paris.
World Organisation for Animal Health (OIE), 2008b. Varrosis of honey bees. OIE Terrestrial Manual, Chapter 2.2.7. OIE, Paris.
World Organisation for Animal Health (OIE), 2008c. American foulbrood of honey bees (infection of honey bees with Paenibacillus larvae. OIE Terrestrial Manual, Chapter 2.2.2. OIE, Paris.
World Organisation for Animal Health, 2016. American foulbrood of honey bees (infection of honey bees with Paenibacillus larvae). OIE Terrestrial Manual, Chapter 2.2.2. OIE, Paris.
Woyke J, 1971. Correlations between the age at which honeybee brood was grafted, characteristics of the resultant queens, and results of insemination. Journal of Apicultural Research, 10, 45–55.
Wu JY, Anelli CM and Sheppard WS, 2011. Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity. PLoS ONE, 6, e14720.
Xue XF, Zhou JH, Wu LM, Fu LH and Zhao J, 2009. HPLC determination of adenosine in royal jelly. Food Chemistry, 115, 715–719.
Yáñez KP, Martín MT, Bernal JL, Nozal MJ and Bernal J, 2014. Trace analysis of seven neonicotinoid insecticides in bee pollen by solid–liquid extraction and liquid chromatography coupled to electrospray ionization mass spectrometry. Food Analytical Methods, 7, 490–499.
Yang E, Chuang Y, Chen Y and Chang L, 2008. Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). Journal of Economic Entomology, 101, 1743–1748.
Young HJ and Stanton ML, 1990. Influences of floral variation on pollen removal and seed production in wild radish. Ecology, 71, 536–547.
Zacepins A and Karasha T, 2013. Application of temperature measurements for the bee colony monitoring: a review. Proceedings of the 12th International Scientific Conference 'Engineering for Rural Development', Jelgava, Latvia, pp. 126–131.
Zacepins A, Brusbardis V, Meitalovs J and Stalidzans E, 2015. Challenges in the development of precision beekeeping. Biosystems Engineering, 130, 60–71.
Zulian G, Maes J and Paracchini ML, 2013. Linking land cover data and crop yields for mapping and assessment of pollination services in Europe. Land, 2, 472–492.

Glossary

Apiary	Defined by the location and the total number of hives at one site belonging to the beekeeper.
Bee feed	Resources, such as pollen, nectar, honey or honeydew, which are collected by bees or provided by a beekeeper for consumption by bees.
Bee inspector	Person with a high level of expertise in bee health and beekeeping management practices.
Beekeeper	The person managing the honeybee colony throughout the year.
Chemical control contaminant	Contaminant originating from pesticides, veterinary medicines (used by a beekeeper and/or livestock farmer) or antibiotics.
Colony	A colony of managed honeybees, defined as an *Apis mellifera* bee population kept by a beekeeper with the presence of a given queen. Replacing the queen by a natural process or by a beekeeper is considered to result in a new colony because it changes the genetics of the population.
Colony attributes	Multidimensional characteristics that are an integral part of the health status of a managed honeybee colony. Can only be assessed indirectly.
Colony outputs	Multidimensional characteristics expressing the productivity of a managed honeybee colony from the perspective of human interest. Can be assessed both directly both indirectly.
Contaminant	Substances (i.e. chemical elements and compounds) or groups of substances that are toxic, persistent and liable to bioaccumulate, and other substances or groups of substances that give rise to an equivalent level of concern.
Disease	A bee showing clinical signs is considered to be diseased.
Ecosystem service	Ecosystem services are defined in TEEB (2010) as 'the direct and indirect contributions of ecosystems to human well-being'.

Ecotype	Locally adapted populations that have evolved traits that confer selective advantage to the population within an ecologically distinct area.
Environmental drivers	Abiotic factors that have been grouped into three categories: soil, weather and climate.
External drivers	Multidimensional characteristics of the colony habitat and management. Can only be assessed indirectly.
Factor	Abiotic or biotic components associated with the external drivers. Multiple factors are used to assess the external drivers.
Foragers	Category of worker bees that perform foraging activities, by identifying sources of food (nectar, pollen) outside the hive and/or bringing it to the hive.
Hive	Non-living parts of the colony. There are several types of hives, varying in size and format. The beehive is the unit containing a honeybee colony used for the production of honey, other apiculture products or honeybee breeding material, and all the elements necessary for its survival (Commission Regulation (EC) No 917/2004).
Indicator	Abiotic or biotic components associated with the colony attributes. Multiple indicators are used to assess the colony attribute.
Infection	The invasion and multiplication of microorganisms, such as bacteria, viruses and parasites, that are not normally present within an animal.
Infestation	The external invasion or colonisation of animals or their immediate surroundings by arthropods, which may cause disease or are potential vectors of infectious agents.
In-hive non-nurses	Category of workers that do not perform nursing (feeding and capping brood) tasks. Non-nurses perform cleaning, nectar reception and storage, and construction tasks, among others.
Nest	Lower box of the hive. If a screen ('queen excluder') is placed between the lower and upper boxes of the hive to prevent the queen passing to the upper boxes, only the nest will contain brood. Honey is typically not harvested from this section.
NUTS	The NUTS classification is a hierarchical system for dividing up the economic territory of the EU
Pest	Any unwanted and destructive insect or other animal that attacks food, crops or livestock.
Predictor	Any type of variable used to predict health; may be an environmental driver or beekeeping management practice.
Preimaginal cells	Cells containing brood (eggs, larvae and pupae).
Provisioning service	Provisioning ecosystem services are the products obtained from ecosystems, such as food, fresh water, wood, fibre, genetic resources and medicines (The Economics of Ecosystems and Biodiversity (TEEB), 2010).
Regulating service	Regulating ecosystem services are defined as the benefits obtained from regulating ecosystem processes, such as climate, natural hazards, water purification and waste management, pollination or pest control (The Economics of Ecosystems and Biodiversity (TEEB), 2010).
Resource providing unit (RPU)	Defined in terms of the environmental components or units responsible for the genesis and regulation of the resources for a colony. The shape and the area of the RPU are defined by the maximum foraging distance reached by the bees of a given colony in all the possible directions starting from the hive. The simplest assumption is that RPU has a round shape with the centre in the hive; different shapes can be hypothesised according to the characteristics of the landscape (e.g. the presence of large water bodies). The structural (e.g. position and dimension of different crops) and functional (e.g. productivity in terms of pollen of the different crops in the RPU) characteristics of the RPU provide information on the availability, type, amount and accessibility of the resources. The RPU can be divided into subunits or patches, which are considered homogeneous areas from a resource production point of view (EUNIS 2007, revised in 2012).
Stressor	Any physical, chemical or biological entity that can induce an adverse response.

Super	Upper box(es) of the hive. If a screen ('queen excluder') is placed between the lower and upper boxes of the hive to prevent the queen from passing to the upper boxes, this section will contain only bee feed. The honey contained in the super is usually harvested by beekeepers.
Supersedure	Natural replacement of the queen by the colony; natural requeening.
Variables	Measurable quantities identified for each indicator and factor. One or more variables are used to estimate each indicator or factor

Abbreviations

ABPV	acute bee paralysis virus
AFB	American Foulbrood
AHAW	Panel on Animal Health and Welfare
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
BMP	beekeeping management practices
BQCV	black queen cell virus
CBPV	chronic bee paralysis virus
DWV	deformed wing virus
EFB	European Foulbrood
EURL	European Union Reference Library
HIS	Health Status Index
LOD	limit of detection
LOQ	limit of quantification
MANOVA	multivariate analysis of variance
MS	mass spectrometry
MUST-B	Multiple Stressors in Bees
NUTS	Nomenclature of territorial units for statistics
PCR	polymerase chain reaction
PLS	partial least squares
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
RPU	resource providing unit
SBV	Sacbrood Virus
SHB	Small hive beetle
TOR	Terms of Reference
WG	Working group

Appendix A – Examples of European studies monitoring bee health

Table A.1 provides some examples of studies monitoring honeybee health in Europe, with a rough indication of included indicators and factors (i.e. level of detail of the assessment is not specified). The list is not complete and MS have often more activities on bees than those covered by the referred studies.

Table A.1: Examples of European studies monitoring honeybee health

		Examples of large field surveys in Europe			
		COLOSS	**EPILOBEE**	**German Bee Monitoring Project**	**BeeNet/ APENET**
Objective	Link between one indicator and explanatory variables	Y	–	Y	Y
	Link between several indicators and explanatory variables	–	–	–	–
Assessment of at least one indicator/ factor related to	Queen performance	Y	–	Y	Y
	Demography	–	Y	Y	Y
	In-hive products	–	–	Y	Y
	Behaviour and physiology	–	–	–	Y
	Disease, infection and infestation	Y	Y	Y	Y
	Resource Providing Unit	Y	Y	–	Y
	Environmental drivers	–	–	Y	Y
	Beekeeping management practices	Y	Y	Y	Y
	Pollination services	–	–	–	–
	Harvested bee products	–	–	–	Y

Y: yes; –: no.

Appendix B – Categorisation of identified indicators and factors

B.1. Colony attributes

Table B.1: Analysis indicators related to queen performance

Indicator (definition)	Criteria	Rationale	Score[(a)]
Presence of a queen (presence of a queen in the hive)	Variables	1) Visual detection of the queen 2) Visual detection of 1-day-old eggs	
	Evidence link with bee health	1 and 2) Without a queen or the ability of the colony to produce a new queen, the colony will surely die (Winston, 1991)	**H**
	Technical feasibility	1 and 2) Currently routinely applied: checking for the presence of the queen is routine for any beekeeping inspection (Büchler et al., 2013). It is recommended that the queen is labelled: not only does marking the queen help in finding her in the hive, but it will also facilitate determining the age of the queen (see Tables C.1 and C.2) (Human et al., 2013)	**H**
	Priority	One of the most important indicators of bee health because the queen is crucial for the demography of the colony	**H**
Longevity of the queen (months that the queen has been alive)	Variables	1) Age of the queen	
	Evidence link with bee health	1) The age of the queen has a big influence on the resultant health of the colony because it affects her egg-laying ability and the likelihood of her being replaced (Tarpy et al., 2000)	**H**
	Technical feasibility	1) Currently routinely applied – can most reliably be determined if the queen is labelled and the beekeeper keeps track of it (Büchler et al., 2013). If a beekeeper replaces the queen regularly, this should be noted because it renders this variable less informative	**H**
	Priority	Knowing the age of the queen is considered very relevant when assessing the potential fecundity of the queen	**H**

Indicator (definition)	Criteria	Rationale	Score[(a)]
Potential fecundity of the queen (queen laying potentially fertilised and viable worker eggs)	Variables	1) Rate of drones (unfertilised eggs) being laid 2) Queen laying viable eggs 3) Number of new queen cells per swarming event 4) Mating success (number of patrilines)	
	Evidence link with bee health	1) A healthy queen should be laying viable fertilised and unfertilised eggs, with appropriate worker/drone proportions and at a rate that is typical for the season (high in early spring and during the beekeeping season, low in late autumn and winter) (Fyg, 1964; Winston, 1991) and from many drones (e.g. she is mated several times) (Mattila and Seeley, 2014; Tarpy et al., 2013). For example, the main purpose of the drones is to mate with new queens, and therefore, they should only be laid during the time of year when mating is likely to happen and at a lower ratio than workers. The presence of too many drones indicates that the queen has become a drone-laying queen or even that workers have taken over egg laying (Fyg, 1964; Oldroyd and Ratnieks, 2000) 2) A healthy queen will lay viable eggs (fertilised and unfertilised), which will be successfully raised by a healthy colony. This should be confirmed with a qualitative assessment 3) The number of new queen cells that are produced is an indicator of colony health because stronger colonies can rear more new queens. However, common beekeeping practice will prevent the formation of queen cells from developing 4) Colonies from queens who are mated with many drones survive better, forage more effectively and thermoregulate better than colonies from singly mated queens (Jones et al., 2005; Mattila and Seeley, 2014; Tarpy et al., 2013)	**H**
	Technical feasibility	1) Not currently routinely applied because it would require precise determination of the number of drone cells and the number of worker cells in the colony 2) Currently routinely applied because it is easy to check whether the colony is successfully raising eggs through all stages of development by identifying eggs, larvae and pupae stages in the hive 3) When the colony is preparing for swarming, a beekeeper can determine the number of queen cells by visual observation (Fell and Morse, 1984); however, this may be more applicable for research, as common beekeeping practices would either replace a failing queen before new queens are reared or would kill supersedure cells 4) Would be difficult to determine because involves sacrifice of the queen and/or patriline assessment of brood	**H**
	Priority	This indicator is very important because it is a determining factor of the demography of the colony.	**H**
Natural queen replacement (rate at which a queen in a hive is superseded (replaced) by the colony)	Variables	1) Rate of natural queen replacement (supersedure)	
	Evidence link with bee health	1) If a colony displays excessive queen replacement (i.e. more frequent than the rest of the apiary), this might indicate that the colony is not healthy or that the queens are of low quality (Page and Peng, 2001)	**H**
	Technical feasibility	1) Can be assessed visually with good record beekeeping methods and made easier if the queen is labelled (Human et al., 2013; Tarpy et al., 2000). However, common beekeeping practices replace queens regularly, making it irrelevant to assess natural queen replacement in these conditions	**H**
	Priority	Easy to assess by a beekeeper keeping track of the queen in each hive. This indicator could be an early sign of impaired health	**H**

Indicator (definition)	Criteria	Rationale	Score[(a)]
Queen mortality (death of a queen)	Variables	1) Rate of queen deaths.	
	Evidence link with bee health	1) In the same way that excessive replacement is indicative of ill health, excessive queen mortality is also (Page and Peng, 2001)	**H**
	Technical feasibility	It is often very difficult to find a death queen in a colony. Furthermore, it is very hard sometimes to determine the reasons for queen mortality. Seeley (1978)	**L**
Size of the queen (weight, dry or wet, of an adult queen, either pre- or post-fertilisation)	Variable	1) Weight of the queen	
	Evidence link with bee health	In general, a good-sized queen is necessary and important for queen health (Woyke, 1971). The weight of the queen is often correlated with the size of her spermatheca (which then determines how much sperm is stored) (Kahya et al., 2008) but not with the number of ovarioles (Hatch et al., 1999; Corbella and Gonçalves, 1982; Jackson et al., 2011)	**H**
	Technical feasibility	Could be routinely measured with appropriate equipment (Büchler et al., 2013)	**H**
	Priority	Weighing the queen requires an effort from the beekeeper beyond that of a standard hive inspection	**L**
Reproductive system (properties of the queen's reproductive system)	Variables	1) Number of ovarioles 2) Weight of ovaries 3) Diameter of spermatheca 4) Number of spermatozoa 5) Sperm mobility and viability in the spermatheca (ability of the sperm stored in the spermatheca to mobilise and to fertilise an egg)	
	Evidence link with bee health	1) The functional significance of ovariole number and how it relates to queen health is unclear (Jackson et al., 2011) 2) The functional significance of the weight of the ovaries is unclear. A high ovary weight may indicate that it contains more ovarioles, or that it contains fewer ovarioles that are more mature. Also, weight of the ovaries is influenced by season and mating status (Kahya et al., 2008) 3) The diameter of the spermatheca is related to the size of the queen and determines how much sperm is stored. In general, a good-sized queen is necessary and important for queen health (Hatch et al., 1999) 4 and 5) The sperm stored in the spermatheca should be of good quality and quantity (Lodesani et al., 2004). Low sperm viability/mobility has been linked to chemical stressors like pesticide exposure, where low sperm quality may often lead to low potential queen fecundity and high queen replacement (Johnson et al., 2013; Williams et al., 2015), and environmental stressors, like severe temperature hikes (Pettis et al., 2016)	**L**
	Technical feasibility	Examining the queen's reproductive system, including the stored sperm, requires sacrificing the queen, which is beyond the scope of standard inspection. Note that consistency in brood pattern, which is covered in demography, also gives a measure of queen's reproductive system.	**L**

(a): The scores are explained in Table 3, Section 2.2.1. The score H-HH is highlighted in green as the indicators with these score are taken forward in TOR3, whereas the other indicators not.

Table B.2: Analysis indicators related to demography of the colony

Indicator (definition)	Criteria	Rationale	Score[(a)]
Brood demography (amount, survival and development of capped brood cells and queen cells in a hive)	Variables	1) Number of worker brood cells [number of capped brood cells within the hive (surface-capped brood cells)] 2) Number of queen cells 3) Number of drone brood cells 4) Brood pattern consistency (qualitative variable)	
	Evidence link with bee health	1) The amount of brood is crucial for the development and survival of the colony (Winston, 1991). The brood must be present during the whole colony development cycle except in winter, or in some particular circumstances. For example, this appears in Cyprus during very hot and dry summers, or in countries where the weather is favourable all year long, for example, in some parts of Greece. The amount of brood follows an annual cycle and the absolute quantities depend in particular on the geographic location of the colony (e.g. differences in food availability and weather conditions) 2) The presence of queen cells has three meanings and must be interpreted in context (Butler, 1975): 2.1) Replacement of a queen that has been lost suddenly by accident. A number of queens are reared in emergency queen cells prepared from normal worker cells, each containing a young female larva (the fertilised eggs can develop into worker or queen, depending on their diet). The consequences for the demography of the colony are positive because its sustainability will be ensured by one of the future queens, when it has been mated) 2.2) Supersedure of a queen that is still heading the colony, and in which the old queen will be eliminated later. Generally, no swarms are produced when colonies supersede their queen (Winston, 1991). The consequences for the demography of the colony are positive because a young queen will replace the previous one that was not in a good condition (e.g. insufficient secretion of pheromones, injured, laying unfertilised eggs, etc.) 2.3) Colony reproduction and swarming (Winston, 1991). The consequences for the demography of the colony are severe because around half of the population of the workers will leave the hive with the queen. By contrast, this event is very positive for the local bee population because an additional colony will be created 3) The presence of too much drone brood (e.g. late in the season) could be a sign that either the queen is dead or infertile (only being able to lay drone eggs). However, the absence, or a very low amount, of drone brood during the reproductive season may be a sign of unhealthy colony 4) The visual observation of the brood pattern consistency (qualitative variable) is interesting to include in a survey. A 'spotty' brood (e.g. > 10% empty cells) may be a sign of a problem of related to sperm quality, or may be indicative of the presence of infectious agents or pests (e.g. *Varroa*)	

Indicator (definition)	Criteria	Rationale	Score[(a)]
	Technical feasibility	1) There are both objective and subjective methods available to evaluate the size of the brood (e.g. Imdorf, et al., 1987; Costa et al., 2012; Delaplane et al., 2013a,b; Odoux et al., 2014; APENET, 2011 – BeeNet, 2014). All these methods allow the beekeeper to evaluate both the worker and drone broods, and also to count all the queen cells 2) Counting the queen cells (drafted or constructed) by observing all sides of the frames is already performed by many beekeepers 3) Checking for the presence of drone brood by observing all sides of the frames is already performed by many beekeepers. The drone brood cells are larger than the worker brood cells and are easily recognisable. High amounts of drone brood may be a sign that the queen is not laying fertile eggs, and very low amounts of drone brood may be a sign of pesticide exposure (Henry et al., 2015) 4) Checking for the presence of a spotty brood pattern is easily applicable in field surveys	**H** **H**
	Priority	This indicator is considered a key determinant of the demography of the colony because brood is crucial for the development and survival of the colony. Assessing this indicator can be implemented by a beekeeper during a standard hive inspection	**H**
Genetic origin (genetic characterisation of the bee subspecies of the in hive)	Variables	Subspecies of the colony individuals	
	Evidence link with bee health	It is generally considered that the best locally adapted bee colonies (climate, food sources, etc.) are subspecies of native bees that have developed over millennia (Meixner et al., 2010; Büchler et al., 2014). There is evidence that much of the variability in the survival of honeybee colonies is connected to the local adaptability, representing differences in climate, vegetation, infestation pressure and colony management (Büchler et al., 2014). Given the intensive trade of queens in Europe (intra- and extra-EC), native bee populations may be more or less introgressed by other subspecies. In a given environment, the health of bee colonies may be influenced by their genetics. For instance, climate change can directly affect flowering dates (see, for example, Root et al., 2003), which might have a negative effect on subspecies whose colonies' development cycle is not fully adapted	**H**
	Technical feasibility	The most accurate method to determine the genetic origin of the colonies and their introgression level is the use of molecular markers, such as microsatellites (Francis et al., 2014). Other methods are less precise (Meixner et al., 2013)	**H**
	Priority	Information on the genetic origin of the colony is interesting to understand the demography of the colony in a given environment but it requires specific molecular techniques that are available in only a few laboratories	**M**
Dead bees (viability of worker bees from a colony at a given time point)	Variables	1) Worker mortality rate 2) Number of dead bees in the vicinity of the hive 3) Number of dead bees in front of the hive (on the flight board) 4) Number of dead bees inside the hive (in the bottom of the hive)	

Indicator (definition)	Criteria	Rationale	Score[(a)]
	Evidence link with bee health	1) The colony mortality rate is obviously an expression of the health of a colony. The workers have a lifespan of several weeks during the active season and several months during the winter (depending on the European geographic region). If workers die prematurely, this has a negative impact on the colony. Workers mortality rate is the sum of mortality within the colony, in front of the colony, and in the fields (the bees which do not return to the hive) 2 and 3) These variables can be assessed by counting the number of bee corpses in the vicinity and in front of the hive. Foragers usually die in the fields, not being expected to find a high number of bees in the hive surroundings. The presence of dead bees around the hive can be indicative of a health problem causing high forager mortality. Dead bees outside the hive are often dispersed by wind or eaten by predators (wasps, etc.), and therefore, the counting of dead bees around the hive can be undervalued 4) It is not expected to find dead bees inside the hive in normal conditions because dead bees inside the hive are usually thrown away by the workers. The presence of dead bees inside the hive is indicative of a health problem	**H**
	Technical feasibility	1) Most bees die in the environment. The most accurate way of assessing their numbers is to use bee counters to assess the numbers of bees that leave the hive and that return, during a defined time, e.g. a day (Struye et al., 1994; Danka and Beaman, 2007). The difference between the two is the number of bees that have not returned to the colony at the end of the day, and therefore, the level of mortality for this day. Another automated way to assess the numbers of ingoing and outgoing bees is to make recordings continuously by means of video cameras coupled to image analysis software. Evaluation of the mortality rate can also be done by using bee samples from the colony under experimental conditions. The labelling of certain workers at birth is a method that tracks their presence in the hive and finally infers their lifetime (that is to say, how many days they can be observed living). The marking of bees at birth can be achieved either by a simple method (paint marks or numbers) or by an automated technique, radiofrequency identification (e.g. Henry et al., 2012) 2, 3 and 4) There are methods used by beekeepers to estimate the number of dead bees within and/or in front of a hive, but underestimation is likely. The mortality of workers in the colony is measured by the number of bee corpses in the bottom of the hive or in the cells of the frames. Complete or partial dead bees inside the hive are removed by undertaker bees which throw them in front of the hive or at a distance of several metres. These dead bees are often dispersed by the wind or eaten by predators (wasps, etc.). The counting of dead bees around the hives can be much undervalued. In some cases, dead bee traps and linen sheets in front the hives are used (Pilling et al., 2013). Estimating and reporting the approximate number of dead bees in the vicinity of the hive, in front of the hive and within a hive by visual observation can be performed by beekeepers. There are no accurate methods to estimate the number of dead bees in the vicinity of a hive	**H**
	Priority	This indicator is considered a key determinant of the health status of the colony during visits to the colony by the beekeeper because it assesses the possible abnormal mortality of bees at that time	**H**

Indicator (definition)	Criteria	Rationale	Score[(a)]
Colony size (number of living adult worker bees in a colony)	Variables	Total number of adult bees	
	Evidence link with bee health	1) The size of the adult bee (worker) population varies according to the annual cycle of the colony. The workers undertake all tasks, except the laying of the eggs which is performed by the queen (Winston, 1991). The number of workers must be appropriate to perform the necessary activities during a given period. Adult workers continue to ensure the development and survival of the colony during the three main periods: 1.1) the harvest season, in order to accumulate food reserves (honey and pollen) 1.2) wintering 1.3) the resumption of activity by the colony at the end of wintering	**H**
	Technical feasibility	Several methods are applied to estimate the amount of workers in a colony, such as weighing or evaluating the surface of frames or combs covered by bees (e.g. Imdorf et al., 1987; Genersch et al., 2010; APENET, 2011; Costa et al., 2012; Delaplane et al., 2013a,b; Pilling et al., 2013; BeeNet, 2014; Odoux et al., 2014). However, all methods could underestimate the real colony size because on opening the hive for evaluations, a certain number of workers fly away and will not be counted, and the methods do not take into account the number of foraging bees that had left the colony at the time of measurement. In order to have the most accurate measurements, it is necessary to estimate their number when most of the population is present in the colony (e.g. at the end of the day)	**H**
	Priority	This indicator is considered a key determinant of the demography of the colony because the number of living adult worker bees affects the capacity of the colony to produce bee products and provide pollination services. Assessing this indicator can be implemented by a beekeeper during a standard hive inspection	**H**
Colony swarming (number of swarms observed from the colony within a given time)	Variables	Swarming rate	
	Evidence link with bee health	In the normal development of a colony, a swarm (one or more per year) can leave the hive to create another colony. This is usually a sign that the colony is densely populated and lacks space to expand within the hive. For the colony, each swarm departure results in a reduction in the number of workers (Seeley, 1995). It should be noted that a decline in the population of workers due to a swarm should not be confused with a cause of abnormal mortality	**H**

Indicator (definition)	Criteria	Rationale	Score[(a)]
	Technical feasibility	The collection of information on this indicator requires that the beekeeper open the hive very frequently to detect signs of swarming and, therefore, it is considered that under normal operations this indicator has low technical feasibility. Using a permanent scale under the hive that tracks the evolution of its weight, or an electronic bee counter that continuously records the number of bees leaving and returning to a colony (Struye et al., 1994) would allow the detection of a colony swarm. A sudden decrease of the weight of the hive in a very short time, or a very high peak in the number of leaving bees (thousands of bees) reflects the departure of a swarm. Videos and sound recordings can also be used to report departuring swarms. If hives are not equipped with such devices, the departure of a swarm can be seen by opening the hive and observing both a significant decrease in the colony size of the workers AND the presence of open queen cells (queens have emerged), AND by the presence of a new queen (Gary, 1976). For this criterion, it is highly recommended that queens are marked when introduced into a colony (queen produced or purchased by the beekeeper), or at any time when the beekeeper sees the queen when opening the hive. It is notable that methods exist to prevent the swarming	**L**
Egg demography (number, survival and development of eggs in a hive)	Variables	1) Egg number 2) Egg survival rate 3) Egg development rate 4) Egg age.	
	Evidence link with bee health	1) During the active season, the presence of eggs in a colony means that the queen is laying; this ensures the development and health of the colony (see Section 3.2.1 'Queen performance'). A small number of eggs in the brood combs, although space is available, may be a warning of a possible problem of the queen. However, in this case, assessment during a single day may not be sufficient to establish a clear diagnosis and must be confirmed in the following days. Abnormal disposition of the eggs in the frames (e.g. brood mosaic) is indicative of pathology. 2) Measurement of egg survival rate could identify health disorders in the colony. Under some circumstances (generally when there is a lack of pollen), the workers can eat a brood, including eggs (Schmickl and Crailsheim, 2001) 3) There is a considerable variability in egg development time depending on the genetic characteristics of bees and environmental conditions (Winston, 1991). Therefore, interpretation of this variable is difficult 4) The age of an egg (1–3 days) is indicated by its position in the cell (that passes from the vertical state to the horizontal state). When they have just been laid, the eggs are vertical, and then they bow over the following days (Winston, 1991). The evaluation of the age of the eggs allows determination of whether the queen is present in the colony that day or has been present in the last 3 days, which is a positive sign for the colony	**H**

Indicator (definition)	Criteria	Rationale	Score[(a)]
	Technical feasibility	1) A simple method is to evaluate the surface of each part of the face of the frames that is occupied by eggs using digital photographs, and then transform this surface area (cm^2) into number of eggs. Another method is use a grid premarked in cm^2 (see Tables C.7 and C.8 for more details) 2) The method consists of photographing frames containing recently laid eggs (< 1 day) and to monitor their presence during the following 3 days. This can also be done using transparent sheets (Schmickl and Crailsheim, 2001). Comparing the number of hatched larvae with the initial number of eggs gives survival rates 3) No assessment is done because the variable is not considered very useful in field surveys. 4) Beekeepers already implement visual inspection of brood combs to detect eggs of different ages	**H**
	Priority	Collection of information on this indicator would require activities by the beekeeper beyond those performed in a standard hive inspection and this indicator is not considered key to assessing the demographic status of the colony	**L**
Larvae demography (number, survival and development of larvae in a hive)	Variables	1) Larval number 2) Larval development rate 3) Larval age 4) Larval survival rate	
	Evidence link with bee health	1) The number of larvae influences the demography of the colony and should be in accordance with the annual cycle. The presence of numerous larvae in a colony means that nurses have sufficient pollen stores to properly fulfil their task of providing food to larvae (jelly production in sufficient quantity and quality). They must also have sufficient honey stores to effectively regulate the temperature of the brood 2) Measuring larval development rate might help to detect health problems in the colony. The appearance of healthy larvae is a positive sign of the development and health of the colony 3) A healthy colony must contain larvae of different ages, which is a sign that the queen has laid normally in the previous days and that the nurses were able to correctly care for larvae (food and heat) 4) Measurement of larval survival rate can detect the alterations in the health of the colony, because if the larvae die, it is a sign that a biological, chemical or physical cause must be involved and must be sought	**H**

Indicator (definition)	Criteria	Rationale	Score[(a)]
	Technical feasibility	1) A simple method is to evaluate the surface of each part of the faces of the combs occupied by the larvae using digital photographs, and then transform this surface (cm^2) into number of larvae. Another method is to use a grid premarked in cm^2 (see Table C.8. for more details). 2) This variable is better suited to experimental laboratory conditions (e.g. in observation hive) than field conditions, as the disruptions caused by frequent opening of a full-size hive would have negative consequences on the rearing of the larvae and may mask the effects which are sought. 3) Visual observation of the combs to see if larvae of all stages are present. 4) The method consists of photographing combs containing 3-day-old eggs (just before the larvae hatch), and replacing the frames in their hives. Just before the capping of the cells, the frames are extracted from the hives and photographed again. Comparing the number of capped cells relative to the initial number of 1-day-old larvae gives the survival rate of the larvae. Another method is to use a grid premarked in cm^2 (see Table C.8 for more details).	**H**
	Priority	Collection of information on this indicator would require activities from the beekeeper beyond those performed in a standard hive inspection and this indicator is not considered key to assessing the demographic status of the colony.	**L**
Pupae demography (number, survival and development of pupae in a hive)	Variables	1) Pupae number 2) Pupae development rate 3) Pupae age 4) Pupae survival rate.	
	Evidence link with bee health	1) The number of pupae influences the demography of the colony and should be in accordance to the annual cycle. The presence of pupae (which are housed in closed cells) is a sign of the good health of the colony. 2) Measuring the pupae's development rate might help to detect health disorders in the colony, because normal development of the pupae is a sign of the emergence of healthy adults. 3) The precise age of the pupae within a brood is difficult to determine, and therefore, its link with the health status of a colony is not known. From this point of view, knowledge of the age of the eggs and larvae is potentially more informative. 4) As for the survival rate of the eggs and larvae, measurement of pupae survival rate can detect health disorders in the colony because if the pupae die, it is a sign that a biological, chemical or physical cause must be involved and must be sought.	**H**

Indicator (definition)	Criteria	Rationale	Score[(a)]
	Technical feasibility	1) A simple method is to evaluate the surface of each part of the face of the combs that is occupied by the pupae using digital photographs, and then transform this surface area (cm^2) into number of pupae. Another method is to use a grid premarked in cm^2 (see Tables C.7 and C.8 for more details). 2) The development of pupae is a slow process (~ 8 days) that occurs in closed cells and is therefore impossible to observe without opening the cells, which is a major source of disturbance of the phenomenon we want to observe. If this measure were to be made, it should identify cells containing nymphs in their first day of pupation and later record the date of the emergence of the corresponding adult. 3) No assessment is done because the variable is not considered very useful in field surveys. 4) The method consists of photographing combs containing old larvae (in open cells) just before the capping of cells, and replacing the combs in their hive. At the end of the pupal period, combs are taken from the hive and photographed again at regular intervals to monitor and quantify emerging adults. Comparing the number of emerging adults relative to the initial number of old larvae would give the pupae survival rate. Another method is to use a grid premarked in cm^2 (see Tables C.7 and C.8 for more details).	**H**
	Priority	Collection of information on this indicator would require activities from the beekeeper beyond those performed in a standard hive inspection and is not considered key to assessing the demographic status of the colony.	**L**
Drone demography (number, survival and development of drones in a hive)	Variables	1) Drone number 2) Drone development rate 3) Drone age 4) Drone survival rate.	

Indicator (definition)	Criteria	Rationale	Score[a]
	Evidence link with bee health	1) Each year, the queen lays hundreds of unfertilised eggs (haploid) that will yield males (drones) (Winston, 1991). These males are of no known benefit to the colony as they only mate with the queen of another colony, but are important for the local bee population (fertilisation of virgin queens from other colonies). Back from their flights, the males do not necessarily return to their original hive. A large number of males in a beehive may mean that the queen lays mainly unfertilised eggs, which can be checked in the brood. If the queen has disappeared, some workers may also lay unfertilised eggs that will yield males (Winston, 1991). In both cases, the colony will disappear after a few weeks because the population of workers is no longer being renewed. 2) Changes in drone development rate will not affect the development of the colony because drones are of no known benefit to the colony as they only mate with the queen of another colony. From the perspective of the local bee population, an abnormal drone development rate may signal problems in their reproductive capacity (number of spermatozoa), which can impact the development and survival of colonies in the local population if fertilisation of the queens is poor. 3) From the standpoint of the health of the colony, this variable is of no major interest because the drones have no role in their own colony as they only mate with the queen of another colony. 4) From the standpoint of the health of the colony, the presence of pathogens on drones (e.g. Nosema) might indicate that the health of other bees in the colony may also be affected.	**H**
	Technical feasibility	1) Visual observation of the presence of many males and/or the presence of only male cells in the brood is already applied by beekeepers. 2–4) No assessment is carried out because the evidence that this indicator is linked with bee health is low.	**H**
	Priority	Collection of information on this indicator would require activities from the beekeeper beyond those performed in a standard hive inspection and this indicator is not considered key to assessing the demographic status of the colony.	**L**
Nurse demography (number, survival and development of nurses in a hive)	Variables	1) Nurse number 2) Nurse development rate 3) Nurse age 4) Nurse survival rate.	

Indicator (definition)	Criteria	Rationale	Score[(a)]
	Evidence link with bee health	1) Worker bees have a temporal basis for the division of labour (Winston, 1991). The nurses are young workers (aged from 3 to ~ 13 days) that feed on large amounts of pollen to make jelly for larval feeding, and eat honey to produce the heat necessary for development of the brood (Winston, 1991). For the health of the colony, their number must be related to the development needs of the brood, for example: 1.1) to increase the population of workers after wintering; 1.2) to replace dead workers during the active season; 1.3) late in the season, to raise workers that will live for several months ('winter bee'). Taking into account the important role of the nurses, it is crucial to evaluate their number, which is directly related to the health of the colony. 2) Nurse bees must have well-developed hypopharyngeal glands, allowing them to make jelly for larvae, in sufficient quantity and quality (Hrassnigg and Crailsheim, 1998). 3) The average age of nurses is between 3 and 13 days, many variations have been noted in the scientific literature, with a maximum age of up to 40 days (Winston, 1991). More important than the age of nurses is the state of development of their hypopharyngeal glands, as well as the level of production of larval jelly (quantity) and its quality. In cases in which the population of foragers is no longer sufficient (e.g. in the case of poisoning in the environment), some nurses may become foragers before the normal age (Huang and Robinson, 1996), reducing the number of nurses available to care properly for the brood, which might hinder brood development and the overall health of the colony (Khoury et al., 2011). Moreover, the transition from preforaging hive activities to foraging behaviour (age of first foraging) has emerged as the key determinant of honeybee worker lifespan (Rueppell et al., 2007). 4) The nurse survival rate is an important indicator of the health of colonies because if they die prematurely, the brood will not receive the care and feeding necessary for its normal development.	**H**

Indicator (definition)	Criteria	Rationale	Score[(a)]
	Technical feasibility	1) Gently opening the hive and lifting the brood frames can allow a brief visual inspection and quantification of nurses. Nurses must cover the brood area to inspect the open brood (eggs and larvae) and feed the larvae only, and to produce heat when necessary (open and operculated brood). If the brood coverage by nurses is not appropriate in terms of the presence of larvae to feed and the outside temperature, it would be considered as an indicator of atypical worker behaviour (see Section 3.2.4) or an insufficient number of nurses, which might be sign of a health problem. A more accurate assessment of the number of nurses may be performed by means of digital photographs, followed by image analysis. Another method is the use a grid premarked in cm^2 (see Tables C.7 and C.8 for more details). 2) There are several methods by which to assess the development of these glands, such as measurement of the diameter of the acini of the gland (Smodiš Škerl and Gregorc, 2010; Hatjina et al., 2013). However, this is mainly done in an experimental setting. 3) Several methods are available to determine nurse age by labelling the bees when they emerge, but this is done only within experimental settings. 4) The labelling of emerging workers allows their later identification, knowledge of the number of days they spend in their activity as nurses (by visual observation or video recordings) and their total lifetime. The marking of emerging bees can be achieved either by a simple method (painting marks or numbers) (see, for example, Von Frisch, 1967) or using an automated technique (e.g. radiofrequency identification, see for example, Streit et al., 2003). This can only be performed under experimental conditions.	**H**
	Priority	An abnormal comb surface covered by nurses is an example of abnormal worker behaviour and will be detected and reported when assessing the indicator 'abnormal worker behaviour'. However, in particular field surveys, differentiation between nurse demography and atypical behaviour might be useful given the important role of nurses in the colony.	**M**
In-hive non-nurses (number, survival and development of non-nurses in a hive)	Variables	1) Total number of non-nurses 2) Development rate of non-nurses 3) Age of non-nurses 4) Survival rate of non-nurses.	

Indicator (definition)	Criteria	Rationale	Score[a]
	Evidence link with bee health	1) Besides the activities of nurses (see above), other activities of in-hive workers are also important to the health of the colony: cleaning, reception and storage of nectar, construction, undertakers, guards, etc. (Winston, 1991). The number of workers in charge of these tasks must be appropriate to the amount of work involved in a particular task, e.g. for the construction of new combs. It is important that these tasks are carried out within the colony but knowledge on the number of in-hive non-nurses is very limited. 2) There is a plasticity in the temporal division of tasks (Robinson et al., 1992; Huang and Robinson, 1996), and thus in the ages at which the workers achieve them, in relation to the needs of the colony. However, some tasks performed by the workers have anatomical–physiological bases, such as the development of glands, for example (Winston, 1991). To build combs, bees secrete wax through glands whose development depends on the age. It is the same for the guard bees that emit an alarm pheromone, production of which changes with age of the bee. A healthy colony must gather a population of workers of different ages, and thus various developmental stages (anatomical and functional), to allow them to carry out the various tasks necessary for the development of the colony. There is great plasticity in the colony for the repartition of the tasks. What is crucial for the development and survival of the colony is the total number of bees (colony size) at any given time. The workers then find a way for the repartition of the tasks (Robinson et al., 1992). 3) Particularly important is the age of the bees at the end of the active season, just before the wintering (Furgala, 1975). It is crucial that the queen has been able to lay worker eggs in sufficient numbers before wintering, and that nurses have had the ability to raise them, so that the size of the colony in winter and the physiological capabilities of the workers can ensure the survival during winter and the restarting of its activity after the wintering. Determining the age of non-nurses is considered relevant before the wintering, but not at other periods of the year. 4) If the survival rate of in-hive non-nurses is reduced, they cannot become foragers, or will be present in insufficient numbers to ensure the amount of food reserves necessary for the development and survival of the colony. In general, if the lifespan of the various categories of workers (including nurses and foragers) is decreased, this leads to additional costs for the colony, in terms of resources, to replace them (e.g. increased egg-laying by the queen, more jelly to produce and distribute).	**H**
	Technical feasibility	Collection of information on this indicator would require activities from the beekeeper beyond those performed in a standard hive inspection and this indicator is not considered key to assessing the demographic status of the colony	**L**
Forager demography (number, survival and development of foragers in a hive)	Variables	1) Total number of foragers 2) Forager development rate 3) Forager age 4) Forager survival rate.	

Indicator (definition)	Criteria	Rationale	Score[a]
	Evidence link with bee health	1) There are two categories of foragers: (i) scouts who find food sources and communicate through dance to (ii) 'simple' foragers (followers) who will exploit the food sources then (Von Frisch, 1967). The honeybee has developed foraging strategies that continuously optimise the harvesting of food (Winston, 1991; Seeley, 1995). For a colony placed in a given environment, the number of potential foragers should allow optimisation of the collection nectar, pollen and honeydew, in order to ensure the development and survival of the colony and consequently the profitability of the professional beekeeper, if any. 2 and 3) Foraging activity is the last task that old bees have to do until they die. Older bees are more experienced than young bees, including when they realise dances (Von Frisch, 1967). If foragers die prematurely, the work performance of the young foragers is lower than that of older ones, which might have a link with the quantity of food (pollen, nectar) harvested. 4) The survival of foragers depends on the landscape context and the foragers' prior knowledge of this landscape (Henry et al., 2012). It is positive for the health of the colony that foragers live as long as possible, first because of their experience accumulated during their flights, and second, to allow sufficient time for nurses to take care of the brood. Early mortality of foragers leads to an imbalance in the different types of bees, especially the nurses (Robinson et al., 1992). As with other categories of bees, if the lifespan of the foragers is reduced, this results in an additional cost to the colony, in terms of resources needed to replace them.	**H**
	Technical feasibility	1) The most accurate way to assess the overall number of foragers is to use electronic bee counters to assess the numbers of bees that leave the hive and that return, during a defined period (Struye et al., 1994; Danka and Beaman, 2007). Another automated way to assess the numbers of incoming and exiting bees would be to make recordings by means of video cameras coupled to image analysis software. There are also less accurate methods available to measure the foraging activity, by visually counting for a specified period (estimating the number of exiting and returning foragers usually within a few minutes) (Delaplane et al., 2013a,b; BeeNet, 2014). Exiting bees are simpler to count because returning bees land with less predictability, but investigators may want to focus on returning bees, for example, if pollen foraging is a parameter of interest (Delaplane et al., 2013a,b). However, accurately determining the number of foragers would require a long observation time. To determine if the colony is successfully foraging, assessing 'foraging activity' is enough. More details are provided in Table C.21 on foraging activity. 2) Feasibility will be low for a survey. However, in case of experimental design, it is possible to label and to follow the activity of the foragers in relation to their age. 3–4) The labelling of emerging workers allows knowledge of how long they perform the activity of foraging and finally their lifespan. The marking of emerging bees can be achieved either by a simple method (painting marks or numbers) or by an automated technique (radiofrequency identification, for example). However, implementation is limited to experimental conditions.	**H**

Indicator (definition)	Criteria	Rationale	Score[a]
	Priority	Collection of information on this indicator would require activities from the beekeeper beyond those performed in a standard hive inspection and would require special equipment to provide accurate data. However, analysis of the forager demography could be included in more detailed studies given their importance for health status of the colony.	**M**

(a): The scores are explained in Table 3, Section 2.2.1. The score H-HH is highlighted in green as the indicators with these score are taken forward in TOR3, whereas the other indicators not.

Table B.3: Analysis indicators related to in-hive products

Indicator (definition)	Criteria	Rationale	Score[(a)]
Type of bee bread (diversity of bee bread composition)	Evidence link with bee health	Once pollen has been brought back to the colony by foragers, the workers treat it to prevent germination, the digestive process begins, and pollen is prepared for long-term storage. When the pollen has been completely processed for storage it is often referred to as 'bee bread' (Winston et al., 1997). However, the terms 'pollen' or 'stored pollen' are often used in publications instead of 'bee bread'. Pollen is the main source of proteins for honeybees and it is necessary for honeybee development. Worker bees (nurses) use pollen to produce royal and worker jelly, which are needed to feed the queen and the larvae. Diversity of pollen is a key factor for a healthy nutritional status (Schmidt, 1984; Schmidt et al., 1987; Di Pasquale et al., 2013; de Groot, 1953). The nutritional contents of pollens can differ between floral species (Roulston and Cane, 2000; Odoux et al., 2012), suggesting that some are of better quality for bees than others	**H**
	Technical feasibility	Palynological analyses are routinely used (Louveaux et al., 1978; Von der Ohe et al., 2004). DNA barcoding is another possible methodology that is in development (Wilson et al., 2010). Analysing the colour of pollen has been described (van der Steen and Brodschneider, 2014), but has limitations for standardisation and harmonisation of its implementation	**H**
	Priority	Assessing the type of bee bread will provide information on the geographical and botanical origin of the pollen and bee bread, and hence, indirectly, on the pollination services provided by the colony	**M**
Amount of bee bread (quantity of bee bread in the hive)	Evidence link with bee health	The quantity of bee bread must be related to the brood's development needs. For example, to increase the population of workers after wintering, replace dead workers during the active season and, late in the season, to raise workers that will live for several months ('winter bees') (Winston et al., 1987). Insufficient quantities of bee bread will affect the health status of the colony	**H**
	Technical feasibility	There are methods to evaluate the quantity of bee bread in the hive, such as digital image analysis and visual estimation (Imdorf et al., 1987; APENET, 2011; Costa et al., 2012; Delaplane et al., 2013a,b; BeeNet, 2014; Odoux et al., 2014). More details are provided in Tables C.13–C.15	**H**
	Priority	It is crucial to assess the amount of bee bread because it represents the protein source of the colony	**H**
Nutritional quality of bee bread (protein content of bee bread)	Variables	Protein content of bee bread [protein content (%), relative amount of amino acids]	
	Evidence link with bee health	Pollen contains 6–28% protein (Stanley and Linskens, 1974). The crude protein content of pollen is an important indicator of its nutritional value. Honeybees require 10 essential amino acids that can only be found in pollen, but may be missing in certain types of pollen (de Groot, 1953). In general, the nutritive value of bee bread to honeybees is higher than that of fresh bee-collected, laboratory-stored or frozen pollen (Brodschneider and Crailsheim, 2010). The shift in the quality of pollen stored in the colony (bee bread) is attributed to microorganisms associated with the honeybee (Vasquez and Olofsson, 2009)	**H**
	Technical feasibility	The Kjeldahl method allows determination of the crude protein content of pollen (Bradstreet, 1954). The quantity and quality of amino acids could be assessed by high-performance liquid chromatography. Beekeepers can take samples and send them to specialised laboratories	**H**
	Priority	Inclusion of the nutritional quality of bee bread is considered to have medium priority for inclusion into field surveys because the protein content is related to the plant origin of the pollen, and thus indirectly to the pollination services provided by the colony	**L**

Indicator (definition)	Criteria	Rationale	Score[(a)]
Chemical contamination of bee bread (concentration of pesticides in bee bread)	Variables	Concentration of chemical contaminants in bee bread (single molecule or multiple molecule analysis)	
	Evidence link with bee health	Multiresidue pesticide analyses have shown that pesticide residues can be present in bee bread or in the pollen sampled in pollen traps [Chauzat et al., 2010; (pollen from pollen traps); Johnson et al., 2010; (bee bread and pollen from pollen traps); Orantes-Bermejo et al., 2010; (bee bread); Lambert et al., 2013; (pollen from pollen traps)]. If pesticide residues are present in bee bread, they may have a negative effect (including sublethal) on bee health	**H**
	Technical feasibility	Multiresidue pesticide analyses are routinely performed by several analytical laboratories The limits of detection and quantification should be the most sensitive possible, in a given context. Beekeepers can take samples and send them to specialised laboratories. It is important to specify that the beebread must be extracted from the cells before analysis (separation of the beebread from the wax)	**H**
	Priority	It is also proposed that data on bee bread pesticide contamination should be collected routinely in field surveys because the frequency of pesticide occurrence and pesticide concentrations have been reported to be higher in pollen than in honey (Chauzat et al., 2011; Lambert et al., 2013).	**H**
Heavy metals contamination of bee bread (concentration of heavy metals in bee bread)	Variables	Concentration of heavy metals in bee bread.	
	Evidence link with bee health	Bees are exposed to heavy metals during foraging (e.g. arsenic, cadmium, selenium, lead) (Bogdanov, 2006; Johnson, 2015). Pollen contaminated with heavy metals has been described (Lambert et al., 2012; Formicki et al., 2013). Cases of heavy metal toxicity to bees have been described in the literature (Bromenshenk et al., 1991; Hladun et al., 2013; review in Johnson, 2015).	**H**
	Technical feasibility	Analytical methods are available (see references row above). Beekeepers can take samples and send them to specialised laboratories.	**H**
	Priority	Because pollen contaminated with heavy metals has been described (Lambert et al., 2012; Formicki et al., 2013), it is recommended that heavy metal contamination of bee bread is measured, in particular in industrialised areas.	**M**
Type of honey (geographical and botanical origin)	Evidence link with bee health	Investigating types of pollen grains, distribution of pollen, flavonoids patterns, aroma compounds and special marker compounds can provide information on the geographical and botanical origin of honey (Anklam, 1998). The type of honey may provide indications on the foraging source and therefore possible exposure to stressors (i.e. pesticides) (Panseri et al., 2014).	**H**
	Technical feasibility	Honey sensor analysis, melissopalynological analysis and physical–chemical methods are available and can be applied routinely (Crane, 1975; Louveaux et al., 1978; Anklam, 1998; Von der Ohe et al., 2004; Cuevas-Glory et al., 2007).	**H**
	Priority	Assessing the type of bee bread and the type of honey will provide information on the geographical and botanical origin of pollen and bee bread, and hence, indirectly on the pollination services provided by the colony.	**M**
Amount of honey, nectar and honeydew (quantity of honey and nectar in the nest)	Variables	Quantity of honey, nectar and honeydew in the nest and the super (which will not be harvested; harvested honey is considered in the indicator 'quantity of harvested honey, Appendix B.9).	
	Evidence link with bee health	Honey is the carbohydrate food of the bees (Crane, 1975). These sugars cover the energy needs of the bees. These requirements are important for the flight activity and heating, for brood rearing and wintering, and many other activities. Bees store honey for use both during the active season and during wintering, to carry out all their tasks and maintain the temperature of the colony at a value that allows survival of the colony. Bees begin to store honey in the main body of the hive ('nest' or 'brood chamber'), in cells around the brood, then if there is not enough space or there is surplus nectar, in the upper part of the hive, the super. Bees can eat the honey of the super, if needed, but it is primarily for beekeepers, who harvest it.	**H**

Indicator (definition)	Criteria	Rationale	Score[(a)]
	Technical feasibility	There are more or less accurate assessment methods to evaluate the quantity of honey in the main body of the hive and in the super, such as digital image analysis and visual estimation (Imdorf et al., 1987; APENET, 2011; Costa et al., 2012; Delaplane et al., 2013a,b; Odoux et al., 2014; BeeNet, 2014). Weighing the super is also possible. More details are provided in Tables C.15–C.17.	H
	Priority	Measuring the amount of honey in the nest and the super is relevant to estimate the available carbohydrates that can be consumed by the bees, particularly in order to survive long periods without a flow of food from the environment.	H
Nutritional quality of honey (carbohydrate content of honey)	Variables	Carbohydrate content of honey	
	Evidence link with bee health	Honey is prepared by the bees from nectar. It is changed from an easily spoiled, thin, sweet liquid to a stable high-density, high-energy food. By inverting the sucrose in the nectar, bees increase the attainable density of the final product, and thus raise the efficiency of the process in terms of calorific density. At the same time, the resistance of the stored product to spoilage by microorganisms is greatly increased (White, 1975). Assessing the nutritional content of honey is not considered to be very relevant because it contains mainly glucose and fructose (85–95% of honey carbohydrates) (White, 1975).	L
Chemical contamination in honey (amount of pesticides in honey)	Variable	Concentration of chemical contaminant in honey (multiresidue/single-residue analysis).	
	Evidence link with bee health	Multiresidue analyses have shown that pesticide residues can be present in honey (Johnson et al., 2010; Orantes-Bermejo et al., 2010; Wiest et al., 2011; EFSA, 2012a; Lambert et al., 2013; Chauzat et al., 2010). Adult honeybees consume honey to perform various tasks that require energy (sugar from honey). If pesticide residues are present in honey, they may have a negative effect (including sublethal) on bee health.	H
	Technical feasibility	Multiresidue pesticide analyses are routinely performed by many analytical laboratories. The limits of detection and quantification should be the most sensitive possible, within a given context. Beekeepers can take samples and send them to specialised laboratories. Samples of honey in the brood chamber are preferred to estimate the honeybee exposition.	H
	Priority	Although the frequency of pesticide occurrence and their concentrations has been reported to be higher in pollen and wax than in honey (Chauzat et al., 2011; Lambert et al., 2013), it is recommended that honey pesticide contamination is also measured under field conditions when possible and especially before wintering.	M
Heavy metals contamination in honey (amount of heavy metals in honey)	Variable	Concentration of heavy metals in honey.	
	Evidence link with bee health	Bees are exposed to heavy metals during foraging (e.g. arsenic, cadmium, selenium, lead) (Johnson, 2015). Honey can be contaminated with heavy metals (review in Bogdanov, 2006; Lambert et al., 2012; Formicki et al., 2013). Cases of heavy metal toxicity to bees have been described in the literature (Bromenshenk et al., 1991; Hladun et al., 2013; review in Johnson, 2015).	H
	Technical feasibility	Analytical methods are available (see references row above). Beekeepers can take samples and send them to specialised laboratories.	H
	Priority	The frequency and/or concentrations of heavy metals in honey seem to be lower than in bee bread, although few data are available.	L

Indicator (definition)	Criteria	Rationale	Score[(a)]
Nutritional quality of jelly (protein and vitamin content of royal and worker jelly)	Variable	Protein and vitamin content.	
	Evidence link with bee health	Royal jelly is the exclusive food of the queen larvae and the basis of the food of the young worker larvae (Winston, 1991). It is also used to feed the adult queen. The jelly is produced by the nurses. The main components are water, proteins, sugars, lipids and other substances, such as vitamins, antimicrobial molecules, trace and mineral elements (Palma, 1992; Stocker et al., 2005). The major royal jelly proteins are thought to be the main factor responsible for the specific physiological role of royal jelly in queen honeybee development, because major royal jelly proteins include numerous essential amino acids, similar to ovalbumin and casein (Schmitzova et al., 1998) or affect cell growth, cell differentiation and cell survival (Salazar-Olivo and Paz-González, 2005). Given these essential functions, the quality of royal jelly produced by the nurse is important for the health of the colony.	**H**
	Technical feasibility	Chemical analysis of jelly has been reported (e.g. Xue et al., 2009; Presoto et al., 2004). Beekeepers can take samples and send them to specialised laboratories.	**H**
	Priority	Information on the nutritional quality of jelly is less informative than the nutritional quality of bee bread.	**L**
Amount of jelly (quantity of royal and worker jelly in the hive)	Variable	Quantity of in-hive jelly.	
	Evidence link with bee health	Larvae are fed by the nurse bee placing a droplet of the brood food secretion on the cell wall or bottom, usually near the larva's mouth. The presence of jelly is required for the development of the larvae. However, the food requirements of larvae have never been precisely determined (Winston, 1991), and therefore, the quantity of jelly eaten by a larva is not known.	**H**
	Technical feasibility	An overall estimate of the presence of jelly on and around the larvae should be sufficient to realise whether the larvae are fed properly (their volume should match their age). It is very difficult to accurately measure the amount of jelly (royal and worker) in cells containing the larvae.	**L**
Propolis quality (composition of propolis)	Variables	Propolis quality.	
	Evidence link with bee health	Propolis (or 'bee glue') is used by bees to block holes and cracks in the hive, cement and strengthen the comb bases, coat the nest cavity with a thin insulating layer and 'embalm' the carcasses of intruders. Propolis may also have some antifungal and antibacterial properties that protect the nest from infection and mould (Winston, 1991; Simone-Finstrom and Spivak, 2010) There is no clear knowledge of propolis quality requirements in relation to bee health.	**L**
Propolis amount (quantity of in-hive propolis)	Variables	Quantity of propolis.	
	Evidence link with bee health	Bees must have within their environment a sufficient amount of propolis to achieve all the tasks mentioned above (see propolis quality). There is a lack of scientific data concerning the quantity of propolis for the bees. As an indicative example, we can report that beekeepers who collect propolis for sale are able to reap 50–350 g in 3–4 months from a colony (http://www.apiservices.com/rfa/articles/propolis.htm). There is no clear knowledge of propolis quantity requirements in relation to bee health.	**L**
Wax quality (composition of wax)	Variable	Quality of wax	
	Evidence link with bee health	Bees secrete wax through their wax glands. Wax is produced by metabolising honey. Also, bees need to eat pollen (proteins) during the first 5–6 days of their life in order to secrete wax (Winston, 1991). The chemical composition of wax is highly complex and variable (Hepburn, 1986; Fröhlich et al., 2000). In order to replace the old combs in their hives, beekeepers	**L**

Indicator (definition)	Criteria	Rationale	Score[(a)]
		can buy commercial beeswax ('wax sheet') for the building of new combs by bees, but it might contain pesticide residues that could affect bee health.	
Amount of wax (quantity of wax in the hive)	Variable	Quantity of wax produced by the colony	
	Evidence link with bee health	Under normal circumstances in a hive, the bees should have enough wax to build or repair the wax combs. It takes 8.4 kg of honey to produce 1 kg of wax (Winston, 1991). Bees cannot produce wax unless there are adequate honey stores in the colony.	**L**
Chemical contamination in wax (concentration of pesticides in wax)	Variable	Concentration of chemical contaminant (single- or multiresidue analysis).	
	Evidence link with bee health	Pesticide residue analyses carried out in hive waxes have shown that they contain many residues of acaricides, insecticides and fungicides (Chauzat et al., 2010; Johnson et al., 2010; Mullin et al., 2010; Orantes-Bermejo et al., 2010; EFSA, 2012a; Wu et al., 2011).	**H**
	Technical feasibility	Multiresidue pesticide analyses are routinely performed by some analytical laboratories. The limits of detection and quantification should be the most sensitive possible, within a given context. Beekeepers can take samples and send them to specialised laboratories.	**H**
	Priority	It is also proposed that data on wax pesticide contamination be collected routinely in field surveys because the frequency of pesticide occurrence and their concentrations have been reported to be higher in wax than in honey (Chauzat et al., 2011; Lambert et al., 2013).	**H**
Heavy metals contamination in wax (concentration of heavy metals in wax)	Variable	Concentration of heavy metals in wax.	
	Evidence link with bee health	Bees are exposed to heavy metals during foraging (e.g. arsenic, cadmium, selenium, lead) (Bogdanov, 2006; Johnson, 2015). Waxes contaminated with heavy metals have been described in industrial areas (Poland; Formicki et al., 2013). Cases of heavy metal toxicity to bees have been described in the literature (Bromenshenk et al., 1991; Hladun et al., 2013; review in Johnson, 2015).	**H**
	Technical feasibility	Analytical methods are available. Beekeepers can take samples and send them to specialised laboratories.	**H**
	Priority	The frequency and/or concentrations of heavy metals in wax seem to be lower than in bee bread, although few data are available.	**L**

(a): The scores are explained in Table 3, Section 2.2.1. The score H-HH is highlighted in green as the indicators with these score are taken forward in TOR3, whereas the other indicators not.

Table B.4: Analysis indicators related to the behaviour and physiology

Indicator (definition)	Criteria	Rationale	Score[(a)]
In-hive recruiting (number of foragers informing nestmates about a particular food source)	Variables	1) Number of nectar recruiters 2) Number of pollen recruiters 3) Number of water recruiters 4) Number of recruiters for other resources.	
	Evidence link with bee health	Providing information to nestmates about a particular food source (recruiting) is fundamental to a successful foraging strategy because it reduces risks and saves energy at the colony level (Dornhaus et al., 2006). 1) The colony is always motivated to collect nectar, and high numbers of nectar recruiters are usually indicative that there is plenty in the landscape to be exploited. However, a low number of recruiters for nectar does not necessarily indicate that there is a dearth of nectar in the landscape. Overall, the number of nectar recruiters is highly influenced by season, brood cycle, colony size, time of day and weather, and is therefore not a useful indicator of health (Seeley, 1989, Seeley, 1995). Although getting enough nutrition to the colony is important, this indicator alone will not give useful data in relation to bee health of a colony. 2) The colony is most motivated to collect pollen when it is actively rearing a brood; however, lower pollen recruitment might also indicate that the colony possesses sufficient storage. Overall, the number of pollen recruiters is highly influenced by season, brood cycle, colony size, time of day and weather, and is therefore not a useful indicator of colony health. (Fewell and Winston, 1992; Seeley, 1995). 3 and 4) The numbers of water/resin/honeydew recruiters will change depending on the season and weather. Therefore, they are not reliable indicators of colony health (Seeley, 1995; Kühnholz and Seeley, 1997).	**L**
Defence behaviour (the propensity of the workers in a colony to attack)	Variable	Number of bees mobilised to attack at any given time.	
	Evidence link with bee health	Bee defensive behaviours vary widely with subspecies, season, weather, temperature and location, and within colony; however, if these are taken into account, any change in the 'normal' defensive state of the colony would be a sign of ill health (Collins et al., 1982).	**H**
	Technical feasibility	Fairly easy to estimate in field surveys by counting the number of stings per unit time on a flag (Hunt, 2007; Hatjina et al., 2014b).	**H**
	Priority	Implementation would require dedicated beekeeper training.	**M**
Detoxification (process by which the honeybee removes toxins)	Variable	Rate at which toxins are detected and removed by honeybees.	
	Evidence link with bee health	Detoxification is an important process for colony health (Mao et al., 2013; Smirle MJ and Winston ML, 1988).	**H**
	Technical feasibility	Challenging to implement widely under field conditions because it involves specialised testing of a sample of bees to determine if detoxification is going on in the bee body (Mao et al., 2011).	**L**

Indicator (definition)	Criteria	Rationale	Score[(a)]
Physiological markers (molecular markers that indicate a physiological process)	Variable	Presence/absence of molecular markers are correlated with important physiological processes. For example, certain physiological markers may be predictive of decreased colony health (Dainat et al., 2012a) or provide insight into the protective benefits of key proteins.	
	Evidence link with bee health	Physiological markers are usually unspecified and do not give direct evidence of a clear link with bee health. Would be challenging to implement widely under field conditions because it requires laboratory testing of markers	**L**
Immune/ behavioural response (physiological and/ or behavioural response to something that is harming a bee)	Variable	1) Presence of markers from known signalling pathways associated with honeybee immune responses 2) Behavioural responses, such as grooming to remove a pest from its own body or that of a nestmate 3) Microbial balance in the hive.	
	Evidence link with bee health	An immune response is a valuable indicator that the colony has been exposed to an infection (Evans and Lopez, 2004; Huang et al., 2012). It can be influenced by the microbial balance in the hive (Anderson et al., 2013).	**H**
	Technical feasibility	Would be difficult to implement in a large-scale survey, because it requires sampling bees, which are sent to a laboratory that evaluates them for markers of immune responses (Di Prisco et al., 2013). Furthermore, markers signalling that immunological pathways are active are generally unspecified, so more knowledge on the bees' immune system is required to allow the correct interpretation of changes in immune parameters.	**L**
Atypical worker behaviour (ectopic or inappropriate behaviours usually not seen for within a given context)	Variables	1) Atypical worker behaviour inside the hive (qualitative) 2) Atypical worker behaviour in the vicinity of the hive (behaviours observed within a 2 m radius of the hive entrance qualitative) 3) Atypical worker behaviour in areas around the hive (beyond a 2 m radius of the hive entrance qualitative).	
	Evidence link with bee health	1) Atypical behaviours of workers, including in-hive workers, are one of the first signs of ill health of the colony (Seeley, 1995). For example, some atypical behaviour may include running quickly over the comb for long periods, trembling (not as part of the tremble dance), failing to cap old larvae or shaking. 2) Atypical behaviours of workers, including in the vicinity of the hive, are one of the first signs of ill health of the colony (Seeley, 1995). For example, an atypical behaviour may include walking around on the ground outside the entrance for long (evidence of paralysis). 3) Atypical behaviour of foragers in the field is an important sign of ill health of the colony (Seeley, 1995). For example, some atypical behaviour may include failure to orient and to navigate successfully from foraging to the hive.	**H**
	Technical feasibility	1 and 2) Currently widely implemented – a beekeeper during routine inspections will be able to notice if the colony is displaying atypical behaviours (Scheiner et al., 2013). The use of sensors, however, is not currently widely used. Sensors to detect vibrational activity may also be used to indicate abnormal behaviours (Bencsik et al., 2015). 3) It would be very difficult to assess worker behaviours in the field, especially as there is no way of knowing the source (home) hive of that bee.	**H**
	Priority	Atypical behaviours of workers are one of the first signs of ill health of the colony and feasibly can be assessed inside the hive and in the vicinity of the hive.	**H**

Indicator (definition)	Criteria	Rationale	Score[a]
Thermoregulation (the process of warming and cooling the hive to maintain a good brood temperature)	Variable	1) The number of workers engaged in heating or cooling behaviours 2) In-hive temperature 3) In-hive relative humidity.	
	Evidence link with bee health	1) Honeybees can regulate the temperature inside the hive; in addition, temperature measurements can help detect events like increased food consumption, the start of brood rearing, the death of the whole colony (Zacepins and Karasha, 2013). Brood volume and winter cluster volume can also be identified by monitoring colony temperature (Zacepins et al., 2015). 2) Numerous studies have demonstrated that either high or low levels of humidity affect the health of the brood and adult bees, either directly, for example at levels < 50% relative humidity in the brood cells no eggs hatch (Doul, 1976), this being particularly relevant for small nuclei, or indirectly by favouring the development of pathologies. For example, raising the humidity from 68% to 87% increases the percentage of brood mummification caused by the chalk brood virus by 8%. *Varroa* destructor and *Nosema* reproductive rate falls with increasing humidity. Thermoregulation and nectar concentration are also intricately linked with humidity levels in the hive (MD Ellis, 2008). 3) Bees normally heat the colony to keep the in-hive temperatures stable for the brood. Additionally, bees may fan to cool the hive. Thermoregulation behaviour will therefore depend on location, weather and season, and would be meaningful evidence of colony health (Seeley, 1985; Fahrenholz et al., 1999).	**H**
	Technical feasibility	1) The low cost of data collection, processing and data transfer from temperature measurement systems facilitates many applications of temperature measurements in beekeeping (Zacepins and Karasha, 2013). 2) Compared with temperature sensors, humidity sensors are more expensive and have to be kept clean and protected from bees because water vapour cannot overcome wax or propolis to reach the sensing element (Zacepins et al., 2015). 3) Specific sensors can be used to measure this factor. 4) Beekeepers can use a temperature probe, but this gives evidence of temperature, not thermoregulation behaviour. Instead, a true measure of thermoregulation behaviour requires real-time assessment of heating and cooling behaviours of the workers. This assessment can be done in experimental settings but not in field surveys.	**L**
Calmness (activity level of bees on the comb)	Variable	The liveliness or movement of the bees on the comb.	
	Evidence link with bee health	Calmness on the comb is a sign that all is well in the colony. Likewise, a lack of calmness, which is an atypical behaviour for some subspecies of bees, is a sign of ill health (Soliman Kamel and Sheppard, 2003). However, calmness is a genetic trait and therefore a meaningless score. Only a change in calmness, in the context of atypical behaviour, may be useful.	**M**
	Technical feasibility	Calmness can be scored based on the level of worker activity on the comb and assessed using a 4-point scale (Hatjina et al., 2014a): 4, very quiet on the honey and brood combs; 3, quiet on combs; 2, bees are moving on the combs; and 1, bees abandon the combs or hives.	**H**
	Priority	Could be implemented by a beekeeper after attending training.	**M**

Indicator (definition)	Criteria	Rationale	Score[(a)]
Colony foraging activity (general worker bee actions bringing resources from the landscape into the hive)	Variables	1) Forager travelling distance 2) Foraging rate 3) Honey yield and pollen content analysis.	
	Evidence link with bee health	1) Honeybees will travel as far as necessary to bring back resources. The mean/maximum distance varies depending on resource availability, but bees can fly up to 10–12 km (Von Frisch, 1967). Because they are flexibly adaptive to exploit the best resources in the landscape, distance gives information about resource availability, such that the further the bees are flying to forage, the fewer resources are available in the immediate vicinity (Von Frisch, 1967; Beekman and Ratnieks, 2000; Couvillon et al., 2014d). Lack of forage in the environment is considered one of the stressors contributing to honeybee decline (Kluser et al., 2010). 2) Foraging rate is the number of foragers departing or leaving from a hive per unit time (e.g. per 10 min). It will be influenced by hive size, season, weather and time of day (Seeley, 1995; Delaplane et al., 2013a,b; Pilling et al., 2013; Riddell Pearce et al., 2013; BeeNet, 2014). Therefore, its value as a comparison is of little use, as it cannot say anything meaningful about the health status. However, any atypical changes in foraging rate of a colony may indicate that the colony is not healthy and provide an important window into colony health. Therefore, any unexpected, colony-level changes in foraging rate are considered under atypical behaviour in vicinity of the hive. Honey yield and honey analysis have been successfully used in a German monitoring project as a measure of foraging behaviour.	**L**
	Technical feasibility	1) Difficult to implement widely – requires the use of observation hives and the decoding of waggle dances. Harmonic radar, which is also expensive, cannot yet cover the distances that bees are able to fly (Riley et al., 2005; Couvillon et al., 2014d). Currently, there are no good ways to automate the dance decoding process, although recent advances (Wario et al., 2015) may be considered an emerging method. 2) Foraging rate fluctuates based on the above-listed factors, so detecting abnormal or atypical changes in rate would require per-hive monitoring during matched weather/season/time of day conditions (Delaplane et al., 2013a,b; Pilling et al., 2013; Riddell Pearce et al., 2013; BeeNet, 2014). Proper monitoring of this variable is only possible in a research setting. Using automated counters and radiofrequency identification tags might facilitate the process, but this is beyond scope of a field survey. 3) Monitoring of honey yield and honey analysis could be implemented to give an idea of foraging behaviour over the year, but this is unspecified. Proper monitoring of this variable is only possible in a research setting.	**L**
Nectar foraging (nectar provision to the colony)	Variables	1) Proportion of successful trips by nectar foragers 2) Type of incoming nectar 3) Nutritional content of incoming nectar (Brix (% sugar) of a forager's crop content). 4) Volume of crop contents (amount of liquid that is carried in a forager's crop as she returns to her hive from a foraging trip). 5) Number of nectar foragers.	

Indicator (definition)	Criteria	Rationale	Score[a]
	Evidence link with bee health	1) If many bees are returning with full crops, it might indicate that nectar resources are available near the hive (Couvillon et al., 2014a,d). 2) The plant type producing the nectar that is collected by nectar foragers. Honeybees use nectar to make honey and can make honey from a variety of sugar solution sources, including man-made syrup. There is little evidence to link nectar type with bee health (Brodschneider et al., 2007). 3) If many bees are bringing lower quality (low sugar content) nectar to the hive, it might indicate that better quality forage is not available in the landscape. Additionally, low sugar content is correlated with the time of year that the bees have to travel far to forage (Seeley, 1995; Couvillon et al., 2014d). 4) If many bees are returning with empty crops, it might indicate that nectar resources are less available near the hive. Additionally, empty crops correlate with a time of year that foraging distances are long (Couvillon et al., 2014a,d). 5) The colony is always motivated to collect nectar, and high numbers of nectar foragers are usually indicative of a lot of nectar in the landscape to be exploited. However, a low number of recruiters for nectar does not necessarily indicate that there is a dearth of nectar in the landscape. Overall, the number of nectar foragers is highly influenced by season, brood cycle, colony size, time of day and weather, and is therefore not a useful indicator of health (Seeley, 1989, 1995).	**H**
	Technical feasibility	Could be challenging to implement widely, as it requires beekeepers to collect returning foragers and then assess the content of their crops (Pankiw et al., 2001; Couvillon et al., 2014a).	**L**
Pollen foraging (pollen provision to the colony)	Variables	1) Weight of incoming pollen 2) Type of pollen 3) Nutritional content of pollen 4) Proportion of trips successful 5) Number of pollen foragers.	
	Evidence link with bee health	1) The need for pollen collection will vary widely depending on colony status, colony size, season, weather and time of day. Therefore, the weight of pollen that is returned to the hive is an unclear indicator of colony health. However, long-term depletion of returned pollen does have severe consequences for the health of the developing brood (Brodschneider and Crailsheim, 2010; Al-Tikrity et al., 1972). 2) Type of pollen is important in that it indicates the plants on which the bees are foraging. The pollen of some plants is of lower quality than that of others. Additionally, plant type will inform about foraging on mass-flowering crops (Brodschneider and Crailsheim, 2010; Fewel and Winston, 1992). 3) The nutritional content of the pollen is important because lower quality pollen (e.g. with low nutritional content) leads to ill health (Brodschneider and Crailsheim, 2010). 4) If pollen foragers are unable to find adequate pollen, the colony's nutritional status will suffer (Brodschneider and Crailsheim, 2010). 5) The number of pollen foragers is regulated at the colony level – if more pollen is needed, then recruitment for pollen foraging will increase. Therefore, the number of pollen foragers at any given time is not a good indicator of health, as it will fluctuate widely depending on colony status, colony size, season, weather and time of day. (Brodschneider and Crailsheim, 2010).	**H**

Indicator (definition)	Criteria	Rationale	Score[(a)]
	Technical feasibility	1) Would be challenging to implement in such a way as to give reliable data because of how widely pollen collection fluctuates. 2) Would require laboratory analysis, but is currently in use – involves sampling pollen in the baskets of returning foragers and then sending it to a laboratory for pollen-type analysis using a microscope or DNA barcoding (Wilson et al., 2010; Odoux et al., 2014; Requier et al., 2014; Bruni et al., 2015; Soares et al., 2015). 3) Would require laboratory analysis, but is currently in use – involves sampling pollen in the baskets of returning foragers and then sending it to a laboratory for nutritional analysis (Keller et al., 2005; Cook et al., 2005; Di Pasquale et al., 2013). 4) Would be difficult to assess in the field – without individually marking bees known to be pollen foragers, there is no way of determining if a particular return trip is unsuccessful or not.	**H**
	Priority	Determining the amount and characteristics of the incoming pollen could be useful to include in a field survey because they are related to characterisation of the in-hive products and indirectly to the provided pollination services. Involvement of a laboratory is required.	**M**
Water foraging (water provision to the colony)	Variable	1) Volume of water 2) Proportion of trips successful 3) Number of water foragers.	
	Evidence link with bee health	1) The water need of the colony will change depending on the season and weather, and will be highly influenced by geography. Often a colony's water need is met by the water that its foragers retrieve incidentally as they collect nectar, because nectar consists largely of water. Sometimes, however, a portion of a colony's foragers must intentionally collect water from streams, ponds and other wet places. Because of the large variation in foraged water volumes, it is not a reliable or useful indicator of colony health (Seeley, 1995; Kühnholz and Seeley, 1997). 2) The number of foragers returning with water in their crop compared with the total number of water foragers. The water need of the colony will change depending on the season and weather and will be highly influenced by geography. Most importantly, the colony regulates its own water needs, so it will be difficult to determine ad hoc what is necessary and then to evaluate if the needs are not being met (Seeley, 1995; Kühnholz and Seeley, 1997). 3) The number of water foragers is regulated at the level of the colony – if more water is needed, usually to cool the hive during thermoregulation – then the number of water foragers will increase. Therefore, the number at any given time is not a good indicator of health because it will fluctuate widely depending on colony status, colony size, season, weather, temperature and time of day.	**L**
Queen pheromone production (pheromones that are emitted at the level of the colony)	Variables	1) Rate of pheromone emission 2) Worker behaviour outcome	
	Evidence link with bee health	1 and 2) Pheromones are important in the biology of queen and workers (Slessor et al., 2005), but there is currently no evidence that pheromone production informs about bee health.	**L**

Indicator (definition)	Criteria	Rationale	Score[(a)]
Queen atypical behaviour (any inappropriate or ectopic queen behaviour)	Variable	The presence or absence of abnormal queen behaviour.	
	Evidence link with bee health	Atypical behaviours by the queen (e.g. queen running around on the comb, queen trying to leave the hive not during a swarming event, queen laying eggs at wrong time of year or laying them inappropriately, e.g. more than one in a cell) are the first sign of ill health of the colony [Seeley, 1995; BeeNet, 2014; COLOSS BEEBOOK, Vols I and II (http://www.coloss.org/beebook)]	**H**
	Technical feasibility	During routine inspections, a beekeeper could note whether the queen is displaying strange or atypical behaviours (Seeley, 1995; BeeNet, 2014).	**H**
	Priority	This can be implemented in field surveys when the beekeeper spends a few additional minutes to assess the behaviour of the queen in detail.	**M**
Feeding brood (nurse behaviour that provides nourishment to the developing bees)	Variable	The number of workers that are engaged in brood-feeding behaviour.	
	Evidence link with bee health	Feeding the brood is a normal behaviour, but is also particular to the time of year (e.g. if brood is being reared). Any disruption in this process would be considered an atypical behaviour and would signal to the beekeeper that something is wrong [Seeley, 1995; BeeNet, 2014; COLOSS BEEBOOK, Vols I and II (http://www.coloss.org/beebook)].	**H**
	Technical feasibility	Would be difficult to assess if there were any disruption to the brood feeding process [Seeley, 1995; BeeNet, 2014; COLOSS BEEBOOK, Vols I and II (http://www.coloss.org/beebook)].	**L**
Comb cleaning (cleaning of cells by nurses, typically done after a new adult emerges from the cell)	Variables	1) The number of workers that are engaged in comb-cleaning behaviour. 2) The proportion of clean versus uncleaned cells.	
	Evidence link with bee health	Comb cleaning is a normal behaviour but there is currently no evidence comb cleaning informs about bee health. It has to be noted that 'comb cleaning' is different from 'hygienic behaviour' because the latter is applied to a dead or dying brood.	**L**
Processing food (turning of nectar into honey)	Variable	The number of workers that are engaged in food processing behaviour	
	Evidence link with bee health	Processing food is a normal behaviour that is required to supply honey to the colony. However, there is currently no evidence that processing food informs about bee health.	**L**
Capping the brood (covering of the brood that occurs at the end of the larval stage)	Variables	1) The number of workers engaged in capping of the brood 2) The amount of capped brood that is found in the colony.	
	Evidence link with bee health	1) and 2) Capping the brood is a normal behaviour in the absence of larvae disease. However, atypical uncapping (or failure of capping for old larvae) of the brood may be an indicator of a problem, i.e. the brood are diseased or unhealthy (Parker et al., 2011).	**H**

Indicator (definition)	Criteria	Rationale	Score[a]
	Technical feasibility	1 and 2) Upon regular inspection of a hive, a beekeeper may note if brood is capped and regular in its placement and shape, which indicates a good number of fertilised (worker) eggs or, instead, if the brood comb is uncapped, which may indicate that the brood is unhealthy, or abnormally shaped, which indicates that it may be drone brood developing in a worker cell (i.e. that the queen has become a drone layer).	**L**
Drones atypical behaviour (incorrect or ectopic behaviours displayed by drones)	Variable	Number of drones engaged in atypical behaviour.	
	Evidence link with bee health	Atypical behaviours of drones, such as leaving the colony not at the correct time for a queen-mating flight, are the one of the first signs of ill health of the colony [Seeley, 1995; BeeNet, 2014; COLOSS BEEBOOK, Vols I and II (http://www.coloss.org/beebook)].	**H**
	Technical feasibility	During routine inspections, a beekeeper could note if the drones are displaying strange or atypical behaviours (Seeley, 1995; BeeNet, 2014).	**H**
	Priority	Could be implemented in a field survey but is less informative than assessing atypical behaviour of workers and/or the queen.	**L**
Hygienic behaviour (a genetically determined behaviour in which workers detect, uncap and remove dead/dying brood)	Variable	Level of hygienic behaviour.	
	Evidence link with bee health	Hygienic behaviour allows honeybees to detect and remove dead and dying brood (Evans and Spivak, 2010; Büchler et al., 2010). However, hygienic behaviour is found in only 10% of unselected colonies (Waite et al., 2003; Perez-Sato et al., 2009; Bigio et al., 2013).	**H**
	Technical feasibility	Could be assessed with the pin-prick assay in the field (Spivak and Gilliam, 1993; Büchler et al., 2014). However, the expression of hygienic behaviour by colonies is significantly affected by brood presence, food influx and season (Bigio et al., 2013), which means that the assay must be performed repeatedly. Lastly, the pin-prick assay requires opening the hive on successive days to determine the removal of dead brood, which makes it challenging, although possible, to assess in the field.	**H**
	Priority	Dedicated training and the reopening the hives on successive days is required.	**M**
Resource receiving (number of in-hive non-nurses specialised in receiving a given resource)	Variable	1) Number of nectar receivers 2) Number of water receivers.	
	Evidence link with bee health	1) The honeybee colony will flexibly regulate the number of receivers depending on the influx of nectar (Seeley, 1995). It is important for the colony that the number of nectar receivers is in relation with the number of nectar foragers, but this will be regulated by the colony. Therefore, this factor is not an indicator of colony health. 2) There are no pollen receivers because pollen foragers unload pollen directly into empty cells.	**L**
Comb building (rate at which the new wax comb is created)	Variable	Rate of comb building.	
	Evidence link with bee health	Comb building is a normal behaviour, but it depends on colony size, season, weather, pheromones, queen status and forage availability. Therefore, it is perhaps not the best indicator of colony health (Pratt, 1998; Ledoux et al., 2001).	**L**

Indicator (definition)	Criteria	Rationale	Score[(a)]
Guarding (inspecting incoming bees and exclude non-nestmates)	Variable	Frequency of guarding errors.	
	Evidence link with bee health	Guarding encompasses inspecting incoming bees, excluding individuals that do not belong to the colony, and alerting other colony workers about intruders. It is a specialised task in that few bees guard, but guarding does not appear to require experience because so few bees remain as guards for very long (Moore et al., 1987). Incorrectly accepting non-nestmates and incorrectly rejecting nestmates are two guarding errors. The frequency of guarding errors is largely dependent on the nectar. Guards will regulate their propensity for errors, whether incorrectly rejecting a nestmate or incorrectly accepting a non-nestmate, based on nectar availability. Therefore, in the autumn when fewer resources are available, bees are less likely to allow in non-nestmates. Because of this adaptation of the colony, guarding errors are not a reliable indicator of colony health (Downs and Ratnieks, 2001).	**L**
Construction of replacement queen cells (creation of cells to raise new, virgin queens)	Variable	The number of new queen cells that are created as part of swarming or supersedure.	
	Evidence link with bee health	Replacement queen cells during the swarming season is a sign that the colony is strong; however, construction of emergency queen cells not during the swarming season is a sign that the queen's health is failing (Seeley, 1985, 1995).	**H**
	Technical feasibility	It would be possible for a beekeeper to keep track of the number of new queen cells (observed during visual inspection of a hive) that are constructed in a colony (Hatch et al., 1999; Tarpy et al., 2000).	**H**
	Priority	Feasible to implement but considered less informative than other indicators describing behaviour and physiology.	**L**
Undertaking (removal of the corpses of dead workers from the hive)	Variable	The number of workers engaged in undertaking behaviour.	
	Evidence link with bee health	Undertaking is a normal behaviour in the colony and will depend on colony size and time of year. Atypically elevated rates, however, may indicate that there is a high level of mortality (Visscher, 1983; Page et al., 1995).	**H**
	Technical feasibility	A beekeeper can check the number of dead bees at the bottom of the hive.	**H**
	Priority	Feasible to implement but considered less informative than other indicators describing behaviour and physiology.	**L**

(a): The scores are explained in Table 3, Section 2.2.1. The score H-HH is highlighted in green as the indicators with these score are taken forward in TOR3, whereas the other indicators not.

Table B.5: Analysis of indicators related to disease, infection and infestation

Indicator (definition)	Criteria	Rationale	Score[(a)]
Disease			
Clinical signs	Evidence link with bee health	The presence of clinical signs in adult bees and/or brood is indicative that infectious agent(s) and/or pest(s) are acting as colony stressors and therefore affecting colony health.	**H**
	Technical feasibility	Checking for the presence of clinical signs can be performed by an experienced beekeeper.	**H**
	Priority	Clinical signs need to be detected as soon as possible in order to implement measures to reduce disease impact and spread of the causative agent.	**H**
Causative agent	Evidence link with bee health	Infectious agents and/or pests can influence the demography and the outputs of the colony and therefore colony health. Disease impact in the colony may depend on the type of agent involved and the infection pressure.	**H**
	Technical feasibility	Diagnostic techniques are available to identify most agents that could cause disease in a colony.	**H**
	Priority	When clinical signs are observed, identification of the agent causing disease is recommended. However, it is not considered feasible to perform identification of the causative agent in all cases where a honeybee colony shows clinical signs.	**M**
Bacteria			
***Melissococcus plutonius* (Presence of *M. plutonius*, the causative agent of European foulbrood (EFB), in the colony)**	Evidence link with bee health	EFB can lead to colony collapse (Forsgren et al., 2013). Different strains of bacteria could lead to differences in virulence in *in vitro* assays (Arai et al., 2012). Clinical signs were reported in 5 of 15 Member States involved in the EPILOBEE study between 2012 and 2014: Belgium, Finland, France, Italy and Latvia (Laurent et al., 2015). In some Member States, hundreds of EFB cases were confirmed annually during several years (e.g. in the UK; Wilkins et al., 2007; Budge et al., 2010). EFB is also endemic to Switzerland where it has been a major problem (Forsgren, 2010) and it could spread to the bordering Member States. The infection with *M. plutonius* is among the World Organisation for Animal Health (OIE)-listed diseases, infections and infestations in force in 2015. EFB is a notifiable disease in some European countries (e.g. in UK; Tomkies et al., 2009).	**H**
	Technical feasibility	Clinical signs are well described but the signs are not unique, and hence, differential diagnosis can be a problem in the field. Microscopy analyses and PCR can be implemented and a field test kit is available (Tomkies et al., 2009). Several real-time PCRs have been developed (Rivière et al., 2013). Methods are described in the OIE Terrestrial Manual (World Organisation for Animal Health (OIE), 2008a,b,c).	**H**
	Priority	Important disease, but reported in only 5 of 15 Member States during EPILOBEE study.	**M**

Indicator (definition)	Criteria	Rationale	Score[(a)]
***Paenibacillus larvae* [Presence of *P. larvae*, the causative agent of American Foulbrood (AFB), in the colony]**	Evidence link with bee health	AFB is the most deleterious disease on honeybee brood (World Organisation for Animal Health, 2013). *Paenibacillus larvae* is able to form extremely tenacious endospores that remain infectious for > 35 years and withstand heat, cold, draught and humidity (Hasemann, 1961). It is the tenaciousness of the spores and the production of extremely high numbers of spores in diseased colonies that make the effective control of AFB so difficult (Genersch, 2010). Honeybee larvae are susceptible to *P. larvae* infection during the early larval stages 12–36 h after egg hatching. At these stages, a dose of 10 spores or fewer may be sufficient for infection of a larva (Genersch et al., 2005). The disease is widespread across Europe: clinical signs were reported in 15 of the 16 countries participating in the EPILOBEE programme in 2013–2014. The prevalence was between 0.5% and 10.5% depending on the country and the time of year (Laurent et al., 2015). Infection with *P. larvae* is among the OIE-listed diseases, infections and infestations in force in 2015. AFB is also defined as a notifiable disease in Council Directive 82/894/EEC and Council Directive 92/65/EEC as last amended. The import of bees and queen bees is only possible from an area that is not subject to the prohibition order associated with an occurrence of AFB (Council Directive 92/65/EEC).	**H**
	Technical feasibility	Clinical signs (e.g. matchstick test revealing typical ropiness of dead larvae) can be easily observed in the colony and PCR can be implemented. A rapid lateral flow device test has been commercialised that can be used in the field, but results have to be confirmed by laboratory analyses. Several real-time PCRs have been developed (Rivière et al., 2013). Methods are described in the OIE Terrestrial Manual (World Organisation for Animal Health, 2013).	**H**
	Priority	Infection needs to be detected as soon as possible because the spores are very infectious and extremely tenacious. AFB is the most deleterious disease on honeybee brood.	**H**
***Pseudomonas apiseptica* (Presence of *P. apiseptica* in the colony)**	Evidence link with bee health	*Pseudomonas apiseptica* causes septicaemia in adult bees. Once established, the disease can be fatal to individual bees but the impact on the overall colony health is low. However, septicaemia is not considered a serious hazard for the colony and data on its distribution are lacking (Morse and Flottum, 1997).	**L**
***Spiroplasma apis* (Presence of *S. apis* in the colony)**	Evidence link with bee health	*Spiroplasma apis* has been identified as the cause of a May-disease-like disorder in honeybee in France (Mouches et al., 1982; Mouches et al., 1983, 1984). The bacterium can kill honeybees when injected or fed (Mouches et al., 1982). Although the bacteria have been detected in other Member States (Ravoet et al., 2013), its impact on honeybee health in Europe is poorly documented.	**L**
***Spiroplasma melliferum* (Presence of *S. melliferum* in the colony)**	Evidence link with bee health	*Spiroplasma melliferum* was first identified in the USA (Clark, 1977). It has also been detected in Europe (Ravoet et al., 2013). This species causes similar clinical signs to S. apis, but is less intense than *S. apis* when fed (Clark, 1978; Mouches et al., 1982). At the colony level, no productivity losses have been observed (Clark, 1977).	**L**

Indicator (definition)	Criteria	Rationale	Score[(a)]
***Other bacteria*(Presence of other bacteria in the colony)**	Evidence link with bee health	1) *Paenibacillus alvei* is a secondary invader of honeybees during EFB outbreaks. This is a saprophytic spore-forming bacterium that germinates and multiplies in bees infected with *M. plutonius*. The bacterium is widespread in Europe but does not participate in the EFB cycle (Morse and Flottum, 1997). 2) *Enterococcus faecalis* is a secondary invader of honeybees during EFB outbreaks. It multiplies in larvae infected with *M. plutonius*. Although the bacterium showed some pathogenicity, it is not involved in EFB development (Morse and Flottum, 1997). 3) *Achromobacter eurydice* is a secondary invader of honeybees during EFB outbreaks. It multiplies abundantly in larvae infected with *M. plutonius* but it can also be found in healthy larvae. The bacterium showed pathogenicity only when mixed with *M. plutonius* (Morse and Flottum, 1997). 4) *Brevibacillus laterosporus* is a secondary invader of honeybees during EFB outbreaks. This is a saprophytic bacterium that multiplies in bees infected with *M. plutonius*. The bacterium does not participate in EFB cycle (Morse and Flottum, 1997). 5) Several *Bacillus* species have been isolated from honeybees (Gilliam and Morton, 1978). However, their effect on honeybee health has not been documented. 6) *Clostridium botulinum* is present in honey (Nevas et al., 2002). The spores can germinate and multiply in dead bees and pupae (Nakano et al., 1994). However, the bacteria are not dangerous for bees.	**L**
Protozoa			
***Malpighamoeba mellificae* (Presence of *M. mellificae* in the colony)**	Evidence link with bee health	The protozoan (amoeba) provokes the amoebic disease. It infects the Malpighian tubules of adult bees. No clear clinical signs are associated with infections by *M. mellificae* (Morse and Flottum, 1997).	**L**
Neogregarines (including *Apicystis bombi*) (Presence of neogregarines in the colony)	Evidence link with bee health	The neogregarines were first detected in honeybees in Europe at the beginning of the 19th century (Morse and Flottum, 1997) and are still detected in some surveys (Ravoet et al., 2013). They are found in the intestinal tract of honeybees. It has been stated that they can cause serious losses in apiculture, but there is little evidence that they cause any measurable damage on infected honeybee colonies (Morse and Flottum, 1997).	**L**
***Other protozoa*(Presence of *other protozoa* in the colony)**	Relevance infection	1) *Crithidia mellificae* was the only trypanosomatid species found in honeybees (Langridge and McGhee, 1967; Ruiz-González and Brown, 2006; Ravoet et al., 2013) until the recent description of a new genus and species by Schwarz et al. (2015). It is present in Asia, Australia, the USA and Europe, including several Member States (Ravoet et al., 2013; Cepero et al., 2014b; Runckel et al., 2014; Cersini et al., 2015). It is established in the digestive tract. To date, there is no consensus on whether the trypanosomatid affects the health of honeybee colonies (Runckel et al., 2011, 2014; Ravoet et al., 2013). 2) *Lotmaria passim* is a second trypanosomatid species infecting honeybees. It has been described recently (Schwarz et al., 2015). Its impact on honeybee health is not known.	**L**

Indicator (definition)	Criteria	Rationale	Score[(a)]
Virus			
Acute bee paralysis virus (ABPV)(Presence of ABPV in the colony)	Evidence link with bee health	Acute bee paralysis virus was first described in honeybees from England (Bailey et al., 1964). It is part of a complex of closely related viruses from the Family Dicistroviridae, like IAPV and KBV (Cox-Foster et al., 2007). This virus has been detected in several Member States and is more common than IAPV and KBV in Europe (see as reviews Allen and Ball, 1996; Ribière et al., 2008; de Miranda et al., 2010). When fed, sprayed on or injected into healthy bees it made them become trembling and paralysed within a few days (Bailey et al., 1964). The virus can infect larvae, pupae and adult bees (see for review de Miranda et al., 2010). ABPV commonly occurs at low levels in apparently healthy bee colonies and causes no reliable field symptoms (Aubert et al., 2008) However, several studies have reported that ABPV can be a major cause of mortality in colonies infected with *V. destructor* in several Member States (see as reviews Allen and Ball, 1996; Ribière et al., 2008; de Miranda et al., 2010).	**H**
	Technical feasibility	Molecular methods are available as multiplex PCR (Carletto et al., 2010), conventional PCRs (Tentcheva et al., 2004; Genersch et al., 2010; Francis and Kryger, 2012) or real-time PCRs (Siede et al., 2008; Kukielka and Sanchez-Vizcaino, 2009; Ciglenečki and Toplak, 2012).	**H**
Chronic bee paralysis virus (CBPV)(Presence of CBPV in the colony)	Evidence link with bee health	Chronic bee paralysis virus infects worker bees and induces striking and well-defined clinical signs (Bailey et al., 1964; Ribière et al., 2010), such as periodically occurring trembling symptoms, an inability to fly and bees with distended abdomens (Aubert et al., 2008) The virus is present in all continents, including Europe (Bailey, 1965, 1967; Ribière et al., 2008; Blanchard et al., 2009). An outbreak of CBPV can lead to a severe loss of worker bees and to the collapse of the colony (Allen and Ball, 1996; Ball and Bailey, 1997; Ribière et al., 2010).	**H**
	Technical feasibility	Typical clinical signs can be observed (Ribière et al., 2010). Molecular methods are available for example, multiplex PCR (Carletto et al., 2010). Conventional PCRs (Tentcheva et al., 2004; Blanchard et al., 2008a) and validated quantitative RT-PCRs (Blanchard et al., 2007, 2012) are available.	**H**
	Priority	Assessing infection with DWV is considered mainly relevant when investigating its role in a multifactorial context.	**L**
Deformed wings virus (DWV)(Presence of DWV in the colony)	Evidence link with bee health	Deformed wing virus is closely associated with *V. destructor* (Ribière et al., 2008). It is one of the most implicated predictors of honeybee decline from various studies conducted in several Member States (Bacandritsos et al., 2010; Genersch et al., 2010; Budge et al., 2015). Individual infections with DWV may cause deformation of emerging bees and earlier death in adults, reducing the survival of winter honeybee (Aubert et al., 2008; Dainat et al., 2012b). It has been considered as the most likely candidate responsible for the majority of colony losses (Schroeder and Martin, 2012).	**H**
	Technical feasibility	Clinical signs are easy to observe. Molecular methods are available, such as conventional PCR (Tentcheva et al., 2004; Genersch et al., 2005), multiplex PCR (Carletto et al., 2010) or real-time PCR (Chen et al., 2005; Tentcheva et al., 2006).	**H**
	Priority	Assessing infection with this virus is considered mainly relevant when investigating its role in a multifactorial context.	**L**

Indicator (definition)	Criteria	Rationale	Score[(a)]
Sacbrood virus (SBV) (Presence of SBV in the colony)	Evidence link with bee health	Sacbrood virus is present in every continent, including Europe (Allen and Ball, 1996; Bailey, 1967; Grabensteiner et al., 2001; Tentcheva et al., 2004; Nielsen et al., 2008; Kukielka and Sanchez-Vizcaino, 2009).The virus affects larvae and causes their death. It also multiplies in young adult bees without causing obvious disease and it has been detected in large amounts in dead adult bees in colonies infected with *Varroa* in Poland and Germany (Ball, 1999).	**H**
	Technical feasibility	The clinical signs (sac-like appearance of diseased larvae) are easy to observe (Aubert et al., 2008; Ball et al., 1999). Several molecular methods have been described, such as multiplex PCR (Carletto et al., 2010), conventional PCR (Grabensteiner et al., 2001; Tentcheva et al., 2004) and real-time RT-PCR (Kukielka and Sanchez-Vizcaino, 2009; Blanchard et al., 2014b; Mingxiao et al., 2013).	**H**
	Priority	Assessing infection with this virus is considered mainly relevant when investigating its role in a multifactorial context.	**L**
Other viruses(Presence of other viruses in the colony)	Evidence link with bee health	1) Arkansas bee virus (ABV) was first identified in the USA (Bailey and Woods, 1974). It often occurs with the BBPV (Lommel et al., 1985). There is a lack of data regarding its impact on honeybee health. There is no evidence that it is present in Europe. 2) Apis iridescent virus (AIV) was first isolated from adult *Apis cerana* specimens from India (Bailey, 1976). There is a lack of data regarding its impact on honeybee health. There is no evidence that it is present in Europe. 3) Aphid lethal paralysis virus (ALPV) was first identified on a bird cherry-oat aphid. It has been detected recently in honeybees (Runckel et al., 2011). Its pathogenicity on honeybee health is not clear although it has recently been shown that an ALPV-like virus, detected in Europe, is able to infect honeybees (Granberg et al., 2013) 4) Berkeley bee virus (BBPV) was first identified in the USA (Lommel et al., 1985). It often occurs with the ABV. There is a lack of data regarding its impact on honeybee health. There is no evidence that it is present in Europe. 5) Black queen cell virus (BQCV) was identified for the first time in dead queen larvae and prepupae. However, infectivity tests on larval and adult bees usually failed (Bailey and Woods, 1977). The virus is intimately associated with *Nosema apis* (Ribière et al., 2008). BQCV seems to be prevalent and widespread in Europe. However, the virus does not cause any visible clinical signs of infection in adult bees and there is little information on the impact of the virus in Europe (Aubert et al., 2008; Ribière et al., 2008). 6) Big Sioux River virus (BSRV) was identified for the first time in honeybees in 2011 (Runckel et al., 2011). There is a lack of data regarding its impact on honeybee health. 7) Bee virus X (BVX) was first identified on adult bees in Arkansas and thereafter detected in most countries of Europe (Ball and Allen, 1988; Ellis and Munn, 2005). There are no characteristic clinical signs associated with natural infections of this virus (Bailey et al., 1983).	**L**

Indicator (definition)	Criteria	Rationale	Score[(a)]
		8) Bee virus Y (BVY) was first isolated from dead adult bees in Great Britain (Bailey et al., 1980) and thereafter detected in other Member States (Ellis and Munn, 2005; Varis et al., 1992). The virus is associated with *Nosema apis* (Bailey et al., 1983). There is a lack of data regarding its impact on honeybee health (Ribière et al., 2008). 9) Cloudy wing virus (CWV) is widely distributed throughout Europe (Varis et al., 1992; Allen and Ball, 1996) It infects both brood and adult bees. Data regarding its impact on the health of honeybee colonies are lacking, although its common prevalence and occurrence suggest that it is not highly pathogenic (Ribière et al., 2008). 10) Filamentous virus (FV) was first identified in the USA and thereafter reported in several Member States (Bailey et al., 1983; Varis et al., 1992; Ellis and Munn, 2005). The virus infects adult bees and it is associated with *Nosema apis*. No clinical signs could be observed associated with FV infection (Aubert et al., 2008; Ribière et al., 2008). 11) Israeli acute virus (IAPV) was first isolated from dead bees in Israel (Maori et al., 2007) an thereafter detected around the world, including in some Member States (Blanchard et al., 2008b; de Miranda et al., 2010; Formato et al., 2011; Pohorecka et al., 2011; Granberg et al., 2013). It is part of a complex of closely related viruses from the Family Dicistroviridae, like ABPV and KBV (Cox-Foster et al., 2007). Although IAPV has been shown to be a significant marker of colony collapse disorder, further work is required to elucidate the precise role it plays in this syndrome (de Miranda et al., 2010; Cox-Foster et al., 2007). Although the virus is extremely virulent when injected into adults or pupae, it does not induce any obvious clinical signs at the individual or colony level (de Miranda et al., 2010). 12) Kakugo virus (KV) has been detected in the brains of aggressive worker honeybees. It shows 96% nucleotide identity with DWV (Fujiyuki et al., 2004). There is a lack of data regarding its impact on honeybee health. 13) Kashmir bee virus (KBV) was discovered first in adult *Apis cerana* (Bailey and Woods, 1977). It has since been detected in *A. mellifera* from around the world, including some Member States (de Miranda et al., 2010; Allen and Ball, 1996; Blanchard et al., 2014a; Cersini et al., 2013). It is part of a complex of closely related viruses from the Family Dicistroviridae, like ABPV and IAPV (Cox-Foster et al., 2007). In nature, KBV has been detected in adult bees and brood (Ribière et al., 2008). Infection of KBV has never been associated with any specific clinical signs (Hornitzki, 1987; Aubert et al., 2008). Although the virus is extremely virulent when injected into adults or pupae, it does not induce any obvious clinical signs at the individual or colony level. The virus has been frequently implicated in honeybee colony losses, but further work is required to elucidate the precise role KBV plays in this syndrome (de Miranda et al., 2010; Cox-Foster et al., 2007). 14) Lake Sinai viruses (LSV-1 and LSV-2) were first discovered in the USA (Runckel et al., 2011) and 2 years later, viruses of the same group were identified in two Member States (Granberg et al., 2013; Ravoet et al., 2013). There is a lack of data regarding their impact on honeybee health (Cornman et al., 2012).	

Indicator (definition)	Criteria	Rationale	Score[(a)]
		15) Slow bee paralysis virus (SBPV) was first isolated in Great Britain (Bailey and Woods, 1974), but only limited data are available regarding its distribution outside the UK (Ribière et al., 2008). The virus is closely related to *V. destructor* (Ribière et al., 2008; Santillán-Galicia et al., 2010). It has been associated with colony collapse disorder in Great Britain (Martin et al., 1998) but further investigations are needed in order to specify its role in this phenomenon. It causes no reliable clinical symptoms (Aubert et al., 2008). 16) *Varroa destructor* virus-1 was first observed in *Varroa* mites collected in hives from the Netherlands. The virus is genetically closely related to DWV and KV (Ongus et al., 2004). Several recombinations between DWV and VDV-1 have been described (Moore et al., 2011; Wang et al., 2013). There is a lack of data regarding its impact on honeybee health. 17) *Varroa destructor* Macula-like virus (VdMLV) has been detected in honeybees in Europe (Ravoet et al., 2013). There is a lack of data regarding its impact on honeybee health.	
Fungi			
***Nosema* spp.(Presence of *Nosema* spp. in the colony)**	Relevance infection	Nosema disease caused by *Nosema apis* has been reported as a serious disease of honeybees in temperate climates (Fries, 1993). The disease affects adult honeybees and causes a decrease in the colony's population, colony strength, adult bee longevity and winter survival rates (Coineau and Fernandez, 2007; World Organisation for Animal Health (OIE), 2008a,b,c). Although the aetiological agent is *N. apis*, another microsporidian of the same genus, *Nosema ceranae*, has been implicated in colony population depletion (Higes et al., 2006). *Nosema ceranae* presents a high prevalence in honeybee colonies (Botía et al., 2012; Klee et al., 2007). Its infection also affects honeybee health (Higes et al., 2008; Antúnez et al., 2009; Mayack and Naug, 2009, 2010; Aliferis et al., 2012; Dussaubat et al., 2012, 2013; Chaimanee et al., 2012) and might lead to colony depopulation (Higes et al., 2008). At present, *N. ceranae* is found more frequently than *N. apis* in some European honeybee colonies (Klee et al., 2007; Paxton et al., 2007). Clinical prevalence of nosemosis has been reported in 12 Member States participating to the EPILOBEE programme in 2012–2013 and/or 2013–2014 (Laurent et al., 2015).Regulation: Notifiable disease in some countries (e. g. nosemosis caused by *N. apis* in France).	**H**
	Technical feasibility	Diarrhoea is a clinical sign, which is easy to observe. However, it is only caused by *N. apis*, but *N. ceranae* is nowadays detected more often than *N. apis*. Discrimination between the two microsporidia is difficult through morphological identification. Counting of spores in the gut of bees can be implemented although this technique shows a low reproducibility. A PCR method has been validated (Carletto et al., 2013) and several real-time PCRs have been developed (Rivière et al., 2013). Methods are described in the OIE Terrestrial Manual (World Organisation for Animal Health, 2013).	**H**
	Priority	Nosemosis has been reported in 10 of 15 Member States involved in the EPILOBEE study between 2012 and 2014 (Laurent et al., 2015). When no clinical signs are detected, it is recommended that the presence of *Nosema* spp. Is assessed only in hives that are located in areas where nosemosis has been reported in recent years.	**M**

Indicator (definition)	Criteria	Rationale	Score[a]
***Ascosphaera apis* (chalkbrood)(Presence of *A. apis* in the colony)**	Evidence link with bee health	Chalkbrood, a disease caused by the fungus *Ascosphaera apis* (Williams, 2000; Hornitzky, 2001), was first described in Germany at the beginning of the 20th century. It has been reported in numerous Member States (Garrido-Bailon E et al., 2008). The fungus infects larvae, which become mummified and die. Reduction in the honey crop, foraging capacity and honey production have been reported. However, chalkbrood is considered as a stress-related disease of honeybees, therefore, of minor importance for honeybee health (Morse and Flottum, 1997).	**L**
***Aspergillus* spp. (stonebrood)(Presence of *Aspergillus* spp. in the colony)**	Evidence link with bee health	Several species of *Aspergillus* have been reported on honeybees, *Aspergillus flavus* being the most frequently detected and the most virulent to honeybee (Shoreit and Bagy, 1995; Foley et al., 2014). In any case, stonebrood is rare and is consider of minor importance to beekeepers. The disease was first described in Germany at the beginning of the 20th century. Thereafter, it has been reported in several Member States (Morse and Flottum, 1997). Stonebrood affects larvae, whereas infection of adult bees is rare (Batra et al., 1973). It is generally accepted that pathogenicity only occurs in colonies weakened by other factors (Shoreit and Bagy, 1995).	**L**
Insects			
***Aethina tumida* (Small hive beetle; SHB) (Presence of *A. tumida* in the colony)**	Evidence link with bee health	The coleopteran *Aethina tumida* (Murray, 1867) is a parasite and scavenger of honeybee colonies. Adults and larvae feed on honeybee brood, honey and pollen, thus causing brood death, fermentation of honey and comb destruction (World Organisation for Animal Health, 2013). The SHB was exotic to Europe until September 2014 since when it has been detected in Calabria (Italy) (Mutinelli et al., 2014; Palmeri et al., 2015). Numerous outbreaks were reported in Italy between September and December 2014. In September–November 2015, other outbreaks were reported in the same region in Italia (see Italian NRL website[b]; EURL website[c]). Moreover, no outbreaks have been reported in any other Member State. Infestation with *A. tumida* is among the OIE-listed diseases, infections and infestations in force in 2015. SHB infestation is also defined as a notifiable disease in Council Directive 82/894/EEC and Council Directive 92/65/EEC, as last amended. Detection of *A. tumida* is notifiable and trade in or import of bees into the Community are only possible if bees originate from the surveillance zone, defined as an area of at least 100 km radius which is not the subject of any restrictions associated with the suspicion or confirmed occurrence of SHB and where this infestation is absent (Council Directive 92/65/EEC). Moreover, bees shall only be introduced into the European Union from Third Countries or territories where the presence of *A. tumida* is subject to compulsory notification throughout the whole of the Third Country or territory concerned (EU Regulation No. 206/2010).	**H**
	Technical feasibility	Traps and observation in the hive can be easily implemented (EFSA, 2015). Methods are described in the OIE Terrestrial Manual (World Organisation for Animal Health, 2013).	**H**
	Priority	*Aethina tumida* has only been reported in Italy so far.	**L**
***Achroia grisella* (lesser wax moth) (Presence of *A. grisella* in the colony)**	Evidence link with bee health	*Achroia grisella* (Fabricius, 1794), a lepidopteran, is spread throughout Europe. The moth infects beekeeping materials. In warm climates this infection occurs simultaneously with *Galleria mellonella*. It is generally considered of minor importance to beekeepers (Morse and Flottum, 1997).	**L**

Indicator (definition)	Criteria	Rationale	Score[(a)]
***Galleria mellonella* (Presence of *G. mellonella* in the colony)**	Evidence link with bee health	*Galleria mellonella*, a lepidopteran, is spread throughout the world, including Europe. This is one of the most ruinous pests of honeycombs; however, its destructive activities are more severe in the tropics and subtropics (Morse and Flottum, 1997).	**L**
***Senotainia tricuspis* (Presence of *S. tricuspis* in the colony)**	Evidence link with bee health	*Senotainia tricuspis* has been described in some Member States. The dipteran has been described as the causal agent of honeybee losses (Haubruge et al., 2006). It infects adult bees. Its impact on the health of honeybee colonies is poorly documented.	**L**
***Braula coeca* (Presence of *B. coeca* in the colony)**	Evidence link with bee health	*Braula coeca*, a Diptera ectoparasite, is known only on honeybees. This louse is distributed worldwide. It was first reported in several Member States at the beginning of the 20th century. It can be found on mated queens, drones and workers. It is usually considered a minor pest (Morse and Flottum, 1997).	**L**
***Other insects* (Presence of other insects in the colony)**	Evidence link with bee health	*Apocephalus borealis*, a dipteran previously known to infect bumblebee, has been recently described as infecting honeybees in North America. It was suspected to participate to the colony collapse disorder (CCD) phenomenon (Core et al., 2012). To date, there is no evidence that the fly is present in Europe.	**L**
Mites			
***Varroa destructor* (Presence of *V. destructor* in the colony)**	Evidence link with bee health	*Varroa destructor* is a parasite of adult bees and larvae. Varroosis can lead to colony collapse (World Organisation for Animal Health (OIE), 2008a,b,c). The mite is widespread throughout Europe: clinical signs of varroosis have been reported in 15 of the 16 countries participating to the EPILOBEE programme in 2013–2014 (Laurent et al., 2015). Infestation with *V. destructor* is among the OIE-listed diseases, infections and infestations in force in 2015. Varroosis is also on the list of diseases for which national programmes may be recognised under Council Directive 92/65/EEC of 13 July 1992.	**H**
	Technical feasibility	The mite can be easily detected and identified in the colony, for instance detection of mites during visual inspection of the hive. Methods are described in the OIE Terrestrial Manual (World Organisation for Animal Health (OIE), 2008a,b,c).	**H**
	Priority	*Varroa destructor* is considered an important factor in colony mortality and is widespread in Europe.	**H**
***Acarapis woodi* (Presence of *A. woodi* in the colony)**	Evidence link with bee health	*Acarapis woodi* (Rennie, 1921) is an internal parasite of the respiratory system living and reproducing in the trachea of adult honeybee *Apis mellifera*. The mite has been reported world-wide including in several Member States (Fakhimzadeh et al., 1993; Matheson, 1993; Sammataro et al., 2000). The presence of the mites has been associated with high levels of bee mortality and poor winter survival (Otis and Scott-Dupree, 1992; Villa and Rinderer, 2008; McMullan and Brown, 2009). However, generally the mite does not seem to be a major cause for concern within Europe. Infestation with *A. woodi* is among the OIE-listed diseases, infections and infestations in force in 2015. Acariasis is also on the list of diseases for which national programmes may be recognised under Council Directive 92/65/EEC of 13 July 1992).	**L**

Indicator (definition)	Criteria	Rationale	Score[(a)]
***Tropilaelaps* spp. (Presence of *Tropilaelaps* spp. in the colony)**	Evidence link with bee health	The mite is exotic to Europe. It is a parasite of honeybee brood. It feeds on bee larvae and pupae and can cause brood malformation, death of bees and subsequent colony decline or absconding (World Organisation for Animal Health (OIE), 2008a,b,c). Infestation with *Tropilaelaps* spp. is among the OIE-listed diseases, infections and infestations in force in 2015. It is also defined as notifiable disease in Council Directive 82/894/EEC and Council Directive 92/65/EEC as last amended. Detection of *Tropilaelaps* spp. is notifiable and trade in or import of bees into the Community are only possible if bees originate from an area of at least 100 km radius which is not the subject of any restrictions associated with the suspicion or confirmed occurrence of *Tropilaelaps* spp. and where this infestation is absent (Council Directive 92/65/EEC). Moreover, bees shall only be introduced into the European Union from Third Countries or territories where the presence of *Tropilaelaps* spp. is subject to compulsory notification throughout the whole of the Third Country or territory concerned (EU Regulation No. 206/2010).	**L**
	Technical feasibility	An infestation by *Tropilaelaps* can be recognised either visually on bees or by examining hive debris. Also molecular methods are available for identifying *Tropilaelaps* sp. (World Organisation for Animal Health (OIE), 2008a,b,c).	**H**
	Priority	The mite is not currently present in Europe.	**L**
Other mites (Presence of other mites in the colony)	Evidence link with bee health	*Euvarroa sinhai* is a mite parasitising drone brood of the Asian bee *Apis florea* (Mossadegh, 1991). The mite can also infect *A. mellifera* (Koeniger et al., 1983). Based on the very limited data available, there is no indication of the presence of *E. sinhai* in Europe.	**L**

ABPV: acute bee paralysis virus; CBPV: chronic bee paralysis virus; CCD: colony collapse disorder; DWV: deformed wing virus; OIE: World Organisation for Animal Health; SBV: Sacbrood virus.
(a): The scores are explained in Table 3, Section 2.2.1. The score H-HH is highlighted in green as the indicators with these score are taken forward in TOR3, whereas the other indicators not.
(b): http://www.izsvenezie.com/reference-laboratories/beekeeping/, last accessed 30 August 2016.
(c): https://sites.anses.fr/en/minisite/abeilles/eurl-bee-health-home, last accessed 30 August 2016.

B.2. External drivers

Table B.6: Analysis of factors related to the Resource Providing Unit (RPU)

Factor	Criteria	Rationale	Score[(a)]
Land cover/use	Definition	Land cover/use describes and characterises the foraging area around the colony. Land cover/use is considered in terms of habitat and resources (forage) provided to the bees. It determines the forage availability in quantity and in quality. Following Eurostat's Concepts and Definitions Database (Eurostat, 2016), land cover is defined as: 'the observed (bio) physical cover on the earth's surface. It is that which overlays or currently covers the ground. This description enables various biophysical categories to be distinguished – basically, areas of vegetation (trees, bushes, fields, lawns), bare soil (even if this is a lack of cover), hard surfaces (rocks, buildings) and wet areas and bodies of water (sheets of water and watercourses, wetlands). [...] It is said that Land Cover is 'observed'. This means that observation can be made from various 'sources of observation' at different distances between the source and the earth's surface: the human eye, aerial photographs and satellite sensors'.	
	Variable	1) Surface (m^2) of the habitat. 2) Centroid of each habitat in the Resource Providing Unit (RPU) [geographical coordinates (x, y) detected through GPS]. 3) Land cover types (three levels, see Table C.28): 3.1) classes/categories (non-cropped areas); 3.2) crop/plant species (cropped areas). 4) Floral resources (see Productivity factor): 4.1) Pollen productivity; 4.2) Nectar productivity.	
	Relevance	The surfaces of the habitat need to be estimated in order to define the relative contribution of different source of feed and other resources. The average foraging distance is estimated to 3 km around the colony (see above). It is important to define the relative contribution of different sources of food and other resources. Future pollinator declines seem likely given forecasts of increasing land-use change (Winfree et al., 2009). Land use is characterised by the arrangements, activities and inputs people undertake in a certain land cover type to produce, change or maintain it	**H**

Factor	Criteria	Rationale	Score[(a)]
	Technical feasibility	The calculation of the distance between the hive and a homogeneous unit of resource should be based on aerial photo-interpretation alternatively by accessing database of land use GPS (through the measurement of the perimeter of the habitat). It is possible to evaluate the land cover/use type by GIS tools and/or existing databases, such as LUCAS 2012 (LUCAS Land Use and Cover Area frame Survey, 2012), The Land Use Database of the Netherlands (LGN7), 2013; Morton et al., 2011). Using the complementary results obtained with these following methods, it should be possible to identify the type of flowers visited over time within the RPU: 1) For characterising and describing the land cover at EU level, the CORINE Land Cover (CLC, 2006) defined at three levels might be sufficient, whereas for an assessment at a local or Member State level a fourth level might be needed. 2) The palynological analysis of the pollen within the nectar in the hive will provide information of the type of flowers the foragers have visited. It is important to mention that during their flight the forager bees might lose some pollen, and therefore, this analysis will not provide a complete picture of the visitation activity. 3) The observation of the waggle dance by which forager bees share with the other members of the colony information about the direction and distance to patches of flowers yielding nectar and pollen. The exact type of flowers being visited is still difficult to interpret. Analysis of the waggle dance can only be done within a research setting.Relevant information about the floral resources within the RPU could be derived by assessing pollen and nectar productivity (see factor 'Productivity'; for methods see Table C.29).	**H**
	Priority	To better understand the influence of the land cover on the health of the bees it is important to retrieve the data across the European Union for characterising the variability of the honeybee habitat.	**H**
Agronomic practices			
Chemical control (plant pest control)	Definition	Plant pest control measures consisting of the application of a chemical treatment on a crop to reduce the potential negative effects caused by the pest.	
	Variable	1) Treated crop 2) Applied rate of active ingredient (in kg/ha) 3) Mode of application 4) Time of application.	
	Relevance	Chemical agents used in agriculture act on pests and diseases injurious to plants to ensuring the phytosanitary status of the crops. This might lead to better plant fitness and flower quality, consequently having beneficial effects on honeybee health by the improved nutrition quality. However, they might elicit adverse side-effects on honeybees.	**H**
	Technical feasibility	Although the farmers in the RPU keep track of the pesticides used for their farming activities, it is very complex and unrealistic to retrieve the information for all the different crops that are grown in the RPU and considering all the different farmers that operate within the area. Databases on the trade of the active ingredients within the European Union are available and could be used as a proxy for the pesticide consumption.	**L**

Factor	Criteria	Rationale	Score[(a)]
Type of farming	Definition	Intensive versus extensive agriculture, organic versus conventional farming versus GMO farming.	
	Variables	1) Amount of input per surface 2) Proportion of cultivated land in the RPU 3) Average surface per farmer 4) Type of farming.	
	Relevance	Specifically related to Agri-Environment Schemes, both lack and mixed-evidence are found in literature (Couvillon et al., 2014 a–d).	**H**
	Technical feasibility	Corinne Land cover, Member State land cover available (Kandziora et al., 2013).	**H**
	Priority	Across the EU the use of agricultural land varies depending on the climate conditions at the local, regional and Member State levels.	**L**
Cultural crop practice	Definition	Crop production measures consisting of the application of non-chemical methods for growing the crop including methods to reduce the potential negative effects caused by the pest. For example, the use of nets, burning crop residues, etc.	
	Variables	1) Number of crops per season 2) Cultivation methods 3) Harvesting time (in relation to flowering) 4) Fertirrigation. 5) Presence of hedgerows and managed woodlands 6) Presence of wild flowers in the orchards 7) Hay making (no wild flowers remaining) 8) Tillage (no wild flowers remaining) 9) Crop rotation 10) Overgrazing (no wild flowers remaining) 11) Others.	
	Relevance	In integrated pest management, mechanical plant pest control methods are listed as a way of contributing to a reduction in the use of pesticides, thus with potential benefits to pollinators (although not specifically related to honeybee health). A number of studies show how crop practices might affect visiting pollinators (although far fewer studies focus on honeybee health), e.g. Kuussaari et al. (2011) and Carreck and Williams (2002). Winfree et al. (2009) show that the reduction in bee abundance linked to the habitat loss and fragmentation was statistically significant in systems in which little natural habitat remain. Habitat loss is related to agricultural practices.	**L**
Other pest control activity (human health and livestock)	Definition	Human and animal pest control measures consisting of the application of a chemical treatment to reduce the potential negative effects caused by the pest.	
	Variable	1) Treated target 2) Applied rate of active ingredient (in kg/ha) 3) Mode of application 4) Time of application.	

Factor	Criteria	Rationale	Score[(a)]
	Relevance to understand the context	Honeybees could become a non-target species victim of the insecticides used to control pests, such as: 1) mosquitoes, cockroaches, black flies or other biting insects in residential areas and landfill sites (Davis et al., 2007; Khallaayoune et al., 2013, 2015; Chaskopoulou et al., 2014); 2) sand flies, stable flies or other biting insects. For instance, in some Member States, water from the sheep dip containing insecticides can be sprayed onto fields and allowed to soak into the soil (Cooke et al., 2004).	**L**
Biological control (plant pest control)	Definition	Pest control measure consisting of the application or release of an organism into a crop to reduce the potential negative effects caused by the pest (e.g. Bt).	
	Variable	1) Use of biological control method: Yes or No 2) Applied rate of potentially harmful Biological Agent (kg/ha).	
	Relevance	No side effects are expected on the bees.	**L**
Productivity			
Pollen productivity (floral resources)	Definition	Pollen produced in each subunit of the RPU in unit time (e.g. day or week).	
	Variable	Weight (mg) and quality of pollen produced in each subunit of the RPU in unit time (e.g. day or week).	
	Relevance	A lack of pollen affects the colony, leads to brood reduction, cannibalism and the poor health status of the colony. Some authors have investigated the effects of pollen quality on honeybee development (Wahl and Ulm, 1983; Keller, 2005; Di Pasquale et al., 2013). Others have studied the influence of floral variation on pollen removal and seed production (Young and Stanton, 1990). Moreover, honey and pollen efficiency of buckwheat depends on the biological characteristics of the cultivated varieties (Alekseyev, 1981).	**H**
	Technical feasibility	Should be based on phenological observation or modelling studies (for modelling study see Table C.33). However, measuring pollen resources (i.e. quantity) in the RPU can be measured and quality of pollen by performing field studies (e.g. palynological analysis). Measuring floral resources in the landscape requires three steps: 1. measure the number of open flowers in each habitat at a given point; 2. sample pollen; 3. extrapolate upwards.	**H**
	Priority	Difficulties and resources required for collecting data to develop the appropriate model.	**M**
Nectar productivity (floral resources)	Definition	Nectar produced in each subunit of the RPU in unit time (e.g. day or week).	
	Variable	Volume (mL) and quality of nectar produced in each subunit of the RPU in unit time (e.g. day or week).	
	Relevance	A lack of nectar will lead to weakening and starvation (death) of the colony (e.g. Dietz, 1975). Nectar is the key nutrient in the honeybee colony diet.	**H**
	Technical feasibility	Should be based on phenological observation (field studies, see below) or modelling studies (see Table C.33). These should account for variations in nectar production expected with temperature and time of the day, plant variety, geographic origin, age of the plant, etc. http://aob.oxfordjournals.org/content/74/4/327.full.pdf Measuring floral resources in the landscape requires three steps: 1) measure the number of open flowers in each habitat at a given point 2) sample nectar (including water and energy) 3) extrapolate upwards. However, the quantity of nectar in the RPU can be measured, for instance with calibrated tubes. The quality of nectar in terms of sugar concentration can be measured by using a refractometer	**H**

Factor	Criteria	Rationale	Score[(a)]
	Priority	Difficulties and resources required for collecting data to develop the appropriate model.	**M**
Water availability	Definition	Fresh water available in a given environment (including permanent and non-permanent water bodies).	
	Variable	Surface of freshwater bodies (m^2); presence/absence in the RPU.	
	Relevance	Water is used for three purposes: cooling the inside of the hive during hot summers, preparing the brood food (mixture of pollen, nectar and water) and to dilute crystallised honey during the winter for consumption. Bees can find water in a variety of places including the smallest and most obscure places (e.g. damp rocks, branches, puddles, pond, drops on vegetation, etc.) so finding water is not really critical (also because good beekeeping practices include the addition of a container with water close to the hive during hot days), whereas finding quality water (e.g. some bees can collect chlorinated water in swimming pools) may be critical.	**L**
Honeydew produced by aphids	Definition	Honeydew is a sugar-rich secretion produced by aphids and scale insects feeding on plant sap. Honeybees collect honeydew and process it into honey called 'honeydew honey'. Honeybees collect honeydew from tree-feeding aphid species and from aphid-infested cereal and other crops, such as potatoes (Maurizio, 1985).	
	Variable	Productivity of honeydew dripping from a tree using a dripping-sensitive paper (Dungan et al., 2004) at a specific time during the foraging season and the sugar composition is determined by high-performance liquid chromatography (Hendrix et al., 1992)	
	Relevance	The availability of carbohydrates is important for survival of the colony (see above: Nectar). In some areas, especially forests, honeydew is the main source of carbohydrates (honey production in forest areas is based on honeydew). Honeybees will collect honeydew depending on the availability of other sources of nectar and the amount and quality of sugar in the honeydew (which depends on both the type of aphid species and the host plant; Fischer and Shingelton, 2001).	**H**
	Technical feasibility	In forest areas it is probably easy to check via the honey stores; in non-forest areas it is difficult. When assessing the sugar in honeydew using standard filter paper, errors can be made if filter papers are not properly dried before use in the field (Dungan et al., 2004)	**L**
Plant exudates productivity	Definition	Plant parts that provide guttation water (saps), resin (propolis): • Guttation is caused by increased root pressure and decreased transpiration (Klepper and Kaufmann, 1966). • Propolis is a resinous material collected by honeybees from plant sources, mainly leaf buds and bark exudates of trees. This resin material is processed by bees (masticated with salivary secretions and where other products, such as pollen and beeswax may be added). The final product called propolis can be used for strengthening, cementing, waterproofing and disinfecting the hive (Burdock, 1998).	
	Variable	1) Productivity of guttation water 2) Productivity of resin.	
	Relevance	Plant exudates are relevant in the context of the use of seed treated plants where there could be acute toxicity (aspect covered by contamination factor)The propolis plays an important role in bee health contributing to bees' social immunity (Simone et al., 2009; Simone-Finstrom and Spivak, 2010)	**L**

Factor	Criteria	Rationale	Score[(a)]
Contamination in environmental matrices			
Contamination in bee feed	Definition	Presence of the contaminants in the bee feed of the RPU.	
	Variable	Contaminant concentration (mg/kg) of *i* in the bee feed of the habitat *j* (*I* = imidacloprid, lead, . . .).	
Contamination in nectar	Relevance	Oral uptake of contaminated nectar is one of the main routes of exposure. High pesticide residues in nectar will lead to death of the colony. Detailed information on this exposure route can be found in EFSA (EFSA, 2012a,b, 2013).	**H**
	Technical feasibility	Nectar in treated fields or collected from bees returning to the hive can be collected and can be chemically analysed.	**H**
	Priority	The chemical compounds used in agriculture are not always the same across the European Union. They also vary depending on the crops grown in the RPU. Moreover, chemical analysis can only be performed on a preselection of active ingredients and therefore is not adapted in the context of field surveys performed at the European Union level.	**M**
Contamination in the air	Definition	Presence of chemical contaminants in the air in the RPU.	
	Variable	Parts per million (ppm) or mg/kg of contaminant *i* in the air of habitat *j* (*i* = imidacloprid, lead, . . .)	
	Relevance	Exposure to air contaminated with pesticides can be an important route of exposure for certain groups of pesticides (soil fumigants) if the colony is close to a treated field (see EFSA, 2012a). The effects of pesticide drifting during treatment was addressed in the APENET project, which mentioned in particular the case of the fatal powdering of bees in flight with particulates of neonicotinoid seed coating and the implications of humidity (Girolami et al., 2012), and the lethal aerial powdering of honeybees with neonicotinoids from fragments of maize seed coat (Marzaro et al., 2011).	**H**
	Technical feasibility	Specific sampling is needed for chemical analysis. For this sampling, the timing is crucial and should be accounted for in the field survey protocol.	**H**
	Priority	The chemical compounds used in agriculture are not always the same across the EU. They also vary depending on the crops grown in the RPU. Moreover, chemical analysis can only be performed on a preselection of active ingredients and therefore is not adapted in the context of field surveys performed at EU level. However, data gathered on the air contamination will be only a partial representation of the exposure because the entire RPU should be covered.	**L**
Contamination in puddle water	Definition	Presence of the chemical contaminants in puddle water in the RPU.	
	Variable	Parts per million (ppm) or mg/kg of contaminant *i* in the puddle water of habitat *j* (*i* = imidacloprid, lead, . . .)	
	Relevance	Oral uptake of residues in puddle water may lead to severe effects on individual bees. Information on this can be found in EFSA (2012a, 2013).	**H**
	Technical feasibility	It is possible to take samples from puddle water for chemical analysis.	**H**
	Priority	Although bees prefer the puddle water to permanent water bodies, the availability of puddle water in the RPU varies according to the climatic conditions in the EU. Therefore, it is difficult to gather representative data of the chemical contamination of the puddle water.	**L**

Factor	Criteria	Rationale	Score[(a)]
Contamination in surface water	Definition	Presence of chemical contaminants in surface water in the RPU (excluding puddle water and water on plant surface).	
	Variable	Parts per million (ppm) of contaminant *i* in the surface water of habitat *j* (*i* = imidacloprid, lead, …)	
	Relevance	Oral uptake of residues in surface water will probably not be an issue for pesticides because the risk assessment for aquatic organisms will normally be protective enough for bees consuming the water (EFSA, 2013). Johnson and Pettis (2014) and Main et al. (2014) provide information on the effects on bees of pesticide exposure through surface water.	**L**
Contamination in the plant	Definition	Presence of chemical contaminants on the plant surface in the RPU. Presence of biological contaminants (i.e. Virus) in plants in the resource providing unit.	
	Variable	Parts per million (ppm) or mg/kg of contaminant *i* on the plant surfaces in habitat *j* (*i* = imidacloprid, lead, …) Presence/absence of the virus on the plants.	
	Relevance	Dust layer on the surface of plants that could contain pollutants such as neonicotinoids. Bees are exposed when they consume the water on plants (mainly in the morning). Li et al., (2014) show that a plant-pathogenic RNA virus, tobacco ringspot virus (TRSV), could replicate and produce virions in honeybees, resulting in infections that were found throughout the entire body.	**L**
Contamination in the soil	Definition	Presence of chemical contaminants in the soil in the RPU.	
	Variable	Parts per million (ppm) or mg/kg of contaminant *i* in the soil of habitat *j* (*i* = imidacloprid, lead, …).	
	Relevance	Soil is not a direct route of exposure because honeybees do not come into direct contact with soil frequently neither they ingest soil (no oral exposure). However, soil contamination may provide information on how much and with what the environment is contaminated, especially in agricultural areas where it has been demonstrated that systemic compounds can be transferred in the plants after one or several years (Henry et al., 2015).	**L**
Electromagnetic fields	Definition	Presence of magnetic fields in the RPU.	
	Variable	Intensity of the electromagnetic field in the RPU.	
	Relevance	Electromagnetic fields may affect honeybee behaviour and biology because these insects have magnetite in their bodies that helps them in navigation.	**L**
Predators			
Hornets (e.g. *Vespa velutina* in the colony)	Relevance	The first report of *Vespa velutina nigrithorax* in Europe was published in 2005 (Haxaire et al., 2006). It has been suggested that the hornet was introduced into France before 2004 (Villemant et al., 2006). The yellow-legged hornet originates in Asia where it preys on the native honeybee *Apis cerana* and the introduced honeybee *A. mellifera*. Nowadays, *V. velutina* is spreading throughout Western Europe (Villemant et al., 2011; Monceau et al., 2013). Although data about its impact on honeybee health remain scarce, this predator is of major importance for beekeepers and contributes to colony loss (Monceau et al., 2013).	**H**
	Technical feasibility	*Vespa velutina* can be easily distinguished from the native European hornet *V. crabro*. It is black and yellow with yellow legs, whereas *V. crabro* is brown and brownish. Moreover, *V. velutina* is smaller than *V. crabro* (Monceau et al., 2013).	**H**

Factor	Criteria	Rationale	Score[(a)]
	Priority	This predator is of major importance for beekeepers and contributes to colony losses (Monceau et al., 2013). Collection of data on the presence of *V. velutina* is not recommended to be applied routinely across the EU, but is recommended in field surveys in affected areas.	**M**
Birds (Impact in the colony due to birds)	Relevance	Several species of birds eat bees, including birds present in Europe. Sometimes, birds can be a serious problem for beekeepers and honeybees (Morse and Flottum, 1997). Their impact on honeybee health in the continent is poorly documented.	**L**
Mammals (Impact in the colony due to mammals)	Relevance	1) Mice and rats can damage brood combs if they can gain entrance. They can destroy the frames and wax comb by chewing them to provide room to build their nest, when outside temperatures begin to drop. No data have been found regarding the impact of mice in European honeybee colonies, but experts assume it is a minor problem. 2) Other mammals, such as bears, can cause severe damage in an apiary. Bears feed on honey and the brood and can affect several colonies of the same apiary in one night (Morse and Flottum, 1997). There are bears in very few Member States and their impact on European beekeeping is not documented, but experts assume it is a minor problem.	**L**

(a): The scores are explained in Table 3, Section 2.2.1. The score H-HH is highlighted in green as the factors with these score are taken forward in TOR3, whereas the other factors not.

Table B.7: Analysis of factors related to environmental drivers

Factor	Criteria	Rationale	Score[a]
Weather			
Temperature	Definition	The temperature of the atmosphere represents the average kinetic energy of the molecular motion in a small region and is defined in terms of a standard or calibrated thermometer in thermal equilibrium with the air (GEMET, 2010).	
	Variable	1) Average temperature/day; . . ./month 2) Min temperature/day; . . ./month 3) Max temperature/day; . . ./month 4) Variation in temperature over time (daily/monthly, etc.).	
	Relevance	The activity of bees highly depends on the outside temperature. Bees are not active if the outside temperature is < 10 °C and activity decreases when the temperature is > 30 °C (Hatjina et al., 2014a).	**H**
	Technical feasibility	Methods for measuring temperature are standardised and described in the WMO Guide to Meteorological Instruments and Methods of Observation (World Meteorological Organization (WMO), 2008).Data can be collected through weather stations data sets, e.g. CRU database, GCM data portal.	**H**
	Priority	Factor of EU relevance and easy to collect as they are already available in different databases (Appendix C).	**H**
Relative humidity	Definition	The moisture content in the earth's atmosphere of the resource providing unit (RPU) (GEMET, 2010).	
	Variable	Relative humidity (%), i.e. the proportion of water vapour in terms of mass, volume or amount of substance (moles).	
	Relevance	Relative humidity greatly influences the reproduction of parasites and pathogens that harm honeybees (e.g. *Nosema* spp.) and also affects honeybees' thermoregulation and activity (Southwick and Moritz, 1987; Özgör et al., 2015). Relative humidity has a significant negative correlation with the foraging activity of honeybees (Kaur and Sihag, 1994).	**H**
	Technical feasibility	Methods for measuring relative humidity are standardised and are described in Part 1 12/12 of table 12.4 in the WMO guidance (World Meteorological Organization (WMO), 2008). Data can be collected through weather stations data sets, e.g. MARS and NOAA Database[b], CRU database[c], GCM data portal.	**H**
	Priority	A factor of EU relevance and easy to collect because it is already available in different databases (see Appendix C).	**H**
Total precipitation (rainfall + snow)	Definition	Rain and snow falling in the RPU in a specific period (mm/hr – snow coverage/days).	
	Variable	1) Amount of rain falling on the ground surface (l/m^2/day; . . . /month). 2) Duration of snow coverage in days (United States Environmental Protection Agency (US EPA), 2010).	
	Relevance	In order to define the foraging breaks (during (summer)/autumn/winter/spring) snow cover is a good indicator.Extreme rainfall in temperate climates is linked to bad foraging (Blaschon et al., 1999; Crailsheim et al., 1999; Schmickl and Crailsheim, 2002;. Van der Zee et al., 2015).	**H**

Factor	Criteria	Rationale	Score[a]
		Snow coverage also affects the foraging activity of honeybees. For example, when snow is deep, the bottom boxes may be below the snow line (an entrance to the top box will allow winter flight) (Moeller, 1977).	
	Technical feasibility	Methods for measuring rainfall are standardised and are described in WMO Part II.8–23 guidance (World Meteorological Organization (WMO), 2008). Through collection of data sets via weather stations (use of rain gauges), CRU database, GCM data portal. At MARS and NAOO see earlier comment. Data indicating snow coverage can be consulted for North America (e.g. US EPA, 2010) and for Europe by consulting data from the Snow Climate Monitoring and Modis website[d] (http://www.europeandataportal.eu/data/en/dataset?tags=snow http://modis.gsfc.nasa.gov/). It is also possible to measure snow cover using remote sensing techniques because snow cover area consists of the fraction of the pixel surface covered by snow according to the image taken by the satellite. Scatterometers and radiometers can provide details on snow depth, data to be used at a local level to define whether hives are covered in snow.	H
	Priority	To account for the diversity of the climate and weather conditions in the EU and to account for their effects on the health of honeybee colonies, if data are not available in existing databases it is crucial to gather them in a field survey through weather station data	H
Solar radiation	Definition	Solar radiation on the surface of the resource providing unit.	
	Variable	Solar radiation data provide information on the amount of the sun's energy striking a surface at a region on earth, during a particular period (MJ/m^2 or Langley).	
	Relevance	Meteorological variables have been reported to affect honeybees' defensive and foraging behaviour together with their thermoregulation (Southwick and Moritz, 1987; Kovac et al., 2010) (i.e. thermoregulation of water foraging honeybees – balancing of endothermic activity with radiative heat gain and functional requirements). For example, radiation in the morning and afternoon positively influences flight departures, but when the sun is high it negatively affects it. Burrill and Dietz (1981) studied the response of honeybees to variations in solar radiation and temperature.	H
	Technical feasibility	Solar radiation can be studied through pyranometers. Methods for measuring solar radiation are standardised and are described in Part 1 7/4, table 7.1 of the WMO guidance (World Meteorological Organization (WMO), 2008)	H
	Priority	This information needs to be gathered from the weather stations in the EU in the context of field surveys	H
Wind	Definition	The perceptible natural movement of the air, especially in the form of a current of air blowing from a particular direction.	
	Variable	1) Speed 2) Direction.	
	Relevance	Importance not clearly expressed in the screened literature. Colonies in exposed, windy locations will quickly become weaker because bees at the periphery chill, drop and may eventually starve because the populations no longer can cover adequate stores.	L

Factor	Criteria	Rationale	Score[(a)]
		Wind protection is paramount in overwintering colonies. In nature, bee colonies tend to seek sheltered cavities in which to build their nests, primarily to protect themselves from the chilling effect of winter winds.	
Atmospheric pressure	Relevance	Importance not clearly expressed in the screened literature.	**L**
Soil	Definition	Surface of the earth composed of organic and mineral matter.	
	Variable	1) Available water content 2) Type 3) Nutrient availability.	
	Relevance	Importance not clearly expressed in the screened literature.	**L**
Climate			
Snow cover	Definition	Snow coverage of the RPU.	
	Variable	Extent of snow coverage in the foraging area (%, km^2, ha, etc.) over time (days per year) (United States Environmental Protection Agency (US EPA), 2010).	
	Relevance	This indicator can be used to determine the length of winter and foraging periods (bees will not forage when there is snow cover). When snow is deep, the bottom boxes may be below the snow line (an entrance to the top box will allow winter flight) (Moeller, 1977). However, foraging is unlikely at temperatures below 10 °C.	**H**
	Technical feasibility	In order to define the foraging breaks (during summer/autumn/winter/spring) snow cover is a good indicator. It is possible to measure snow cover through remote sensing techniques because snow cover area consists of the fraction of the pixel surface covered by snow according to the image taken by the satellite (Parajka et al., 2008, 2009; Gafurov and Bardossy, 2009; Gao et al., 2010). Scatterometers and radiometers can provide details on snow depth, data to be used at a local level to define if hives are covered in snow.	**H**
	Priority	This information may have added value, particularly on sunny days when temperatures increase but snow cover might still affect foraging.	**M**
Climate type	Definition	Climate type is defined based on categories described by climate classification systems (e.g. the Köppen climate classification system Kottek et al., 2006)	
	Variable	1) The climate class into which the RPU falls. The coordinates of beehives are required 2) Amount of rain falling on the ground surface over the long term (l/m^2 per month) 3) Average temperature/day in seasons/years 4) Thermal sums (degree days).	

Factor	Criteria	Rationale	Score[a]
	Relevance	The type of climate is a fundamental covariate in any analysis of the type and productivity of the RPU. Moreover, *Nosema ceranae* virulence in Europe is attested to be climate dependent (Gisder et al., 2010). The total amount of heat required, between the lower and upper thresholds, for an organism to develop from one point to another in its lifecycle is calculated in units called degree days (°D). Sometimes called heat units. Temperature controls the developmental rate of many organisms. Plants and invertebrate animals, including insects and nematodes, require a certain amount of heat to develop from one point in their lifecycle to another. This measure of accumulated heat is known as physiological time. Theoretically, physiological time provides a common reference for the development of organisms. The amount of heat required to complete a given organism's development does not vary – the combination of temperature (between thresholds) and time will always be the same. Physiological time is often expressed and approximated in units called degree days (°D). Temperature clearly influences the life cycles of most parasitic species (Bush et al., 2001).	**H**
	Technical feasibility	The use of beehive coordinates is enough to detect in which specific climate class the beehive falls (online database). Average precipitations and temperatures in the EU can be retrieved from different databases.	**H**
	Priority	The climate pattern needs to be clarified for each colony that is scrutinised in the field survey across the EU using, for example, the Köppen climate classification system.	**H**

(a): The scores are explained in Table 3, Section 2.2.1. The score H-HH is highlighted in green as the factors with these score are taken forward in TOR3, whereas the other factors not.
(b): NOAA: United States Department of Commerce, National Oceanic and Atmosphere Administration. Database available online: http://www.esrl.noaa.gov/psd/data/data_faq.html
(c): CRU: University of East Anglia Climatic Research Unit. Database available online: http://www.cru.uea.ac.uk/data
(d): MODIS: Moderate Resolution Imaging Spectroradiometer, provided by the National Aeronautics and Space Administration (NASA). Database available online: http://modis.gsfc.nasa.gov/data/

Table B.8: Analysis of factors related to beekeeping management practices

Factors	Criteria	Rationale	Score[(a)]
Beekeeper characteristics			
Beekeeper category	Definition	The number of managed colonies (i.e. hives) within the apiary and the economic viability (e.g. income) of the beekeeper activity provides information on the beekeeper category (Chauzat et al., 2013). According to the Regulation (EU) No 917/2004, two types of beekeeper are distinguished, professional beekeepers with > 150 hives, and non-professional beekeepers with < 150 hives. However in Europe, there was common consensus among the countries on three beekeeper categories: hobby beekeepers, part-time beekeepers and professional beekeepers (Chauzat et al., 2013).	
	Variables	1) Significant source of income of the activity [Y/N] 2) Hive rental for crop pollination [Y/N] 3) Number of colonies managed 4) Migration activity [Y/N] 5) Production type of the colony (derived from factor below) 6) Production system (organic/conventional); according to Regulation (EC) No 834/2007 and Reguation (EC) No 836/2014) 7) Average production per colony in the previous 5 years (not necessarily applicable to hobbyist beekeepers). The colonies considered for this calculation should be alive and productive (i.e. they were expected to produce) 8) Investment effort trend (size, time and investments), i.e. 0 = reduction, 1 = constant, 2 = increase.	
	Relevance	Deloitte (2013) reports that colony losses lead to economic losses and, due to their unpredictable nature, this source of uncertainty could limit the recruitment of a new generation of beekeepers. Long-term decline in colony numbers and/or and weakening of colonies could be driven by socioeconomic and political pressure on honey production (Smith et al., 2013). Similarly, this aspect could be influenced by specific socioeconomic factors, such as the presence or absence of state subsidies (EASAC Policy Report 26, 2015). Thus, the number of beekeepers and their economic status might be both a cause and an effect of the decline in the number of honeybee colonies (Potts et al., 2010; Deloitte, 2013; Smith et al., 2013). As shown in Van der Zee et al. (2012), in most of the countries under scrutiny, hobbyist beekeepers (managing 1–50 colonies) reported higher losses than practitioners with intermediate numbers of beekeeping operations (51–500 colonies). This relationship between the number of hives managed and the extent of losses effect was also observed in the period 2009–2010, even if it was less pronounced (Van der Zee et al., 2012).	**H**
	Technical feasibility	According to Regulation (EU) No 1308/2013, national programmes for the sector should be drawn up every 3 years with a view to improving the general conditions for the production and marketing of apiculture products. Therefore, an overview of the apiculture sector (e.g. number of beekeepers across the EU and their economic viability status) is available; for this reason, it is considered highly technical feasible. The beekeeper category could be assessed through questionnaire, by asking whether the activity represents the 'significant source of income' for the beekeeper.	**H**

Factors	Criteria	Rationale	Score[a]
	Priority	The beekeeper category allows estimation of the number of beekeepers and their economic status across the EU. This factor and its variables (e.g. number of hives managed) assessed according to beekeeper experience is essential to define an harmonised overview of the beekeeping activity in a field survey across all the Member States.	**H**
Beekeeper experience	Definition	The beekeeper experience is linked to personal beekeeping skills gained through practice and training (e.g. years of practice, qualification obtained knowledge of environmental stressors).	
	Variables	1) Age and gender of the beekeeper. *Training* 1) Number of beekeeping courses attended. (e.g. 0, 1–3, 4–6, 7+) 2) Bee meetings attended. (e.g. 0, 1–3, 4–6, 7+) 3) Qualifications obtained. (e.g. 0, 1–3, 4–6, 7+) 4) Membership of a beekeeping association (low score on its own) 5) Years of practice (e.g. 0, 1–3, 4–6, 7–9, 10+) *Technical abilities* (Efforts are required to find accurate methods that can be applied across the EU) 1) Knowledge of good beekeeping practices 2) Knowledge of bee pests/parasites/diseases biology identification and control methodologies 3) Knowledge and application of strategies (i.e. monitoring effort, use of biotechnical/mechanical control methods) 4) Knowledge of bee biology 5) Knowledge of external drivers 6) Colony survival rate/year.	
	Relevance	The beekeeper experience influences the ability to understand and cope with the health status of the colonies. It also influences beekeeping management practices used in specific scenarios.The ability of the beekeeper to manage the colonies influences honeybee health (EPILOBEE, 2012–2014) and is therefore scored as highly relevant.	**H**
	Technical feasibility	This is already included in currently used questionnaires (NBU questionnaire, 2014; EPILOBEE, 2012–2014) and is therefore considered highly technical feasible.Information on the beekeeping experience could also be determined by the evaluation of other beekeeping management practices variables; for example, the beekeeping effort could be assessed through the number of combs removed/introduced per year, captured in the comb management factor.	**H**
	Priority	Assessment of the beekeeper experience is needed, together with the information on beekeeper category, to specifically train the different beekeepers involved in the surveillance activity to ensure harmonisation across the survey's activities. The experience of the bee inspector for implementation of beekeeping management practices is of EU relevance and has an effect on the bee health (EPILOBEE, 2012–2014).	**H**

Factors	Criteria	Rationale	Score[a]
Colony management			
Replacement of combs with brood	Definition	Introduction or removal of combs with brood from the colony.	
	Variable	Specify quantity of brood, adult bees and feed in the combs managed. The data recorded should be as precise and accurate as possible. However, to facilitate the work from the beekeeper/bee inspector, the survey coordinators could decide to use categories (e.g. month instead of date). 1) Date and number of brood combs introduced per colony/year 2) Date and number of brood combs removed per colony/year. 3) Number of brood combs introduction events/colony/year 4) Number of brood combs removal events per colony/year.	
	Relevance	Combs with brood derived from one colony and inserted into another colony will lead to an increase in colony strength in the receiver colony once the brood emerges and a reduction in colony strength in the donor colony. However, unbalanced brood/adult rate may lead to negative consequences for the receiver colony too. In fact, brood rearing requires nurse bees' time and energy (i.e. royal and worker jelly production and feeding, thermoregulation effort). Brood may contain infectious agents and pests (e.g. AFB, EFB, *Varroa* mites, *Nosema* spp.) that may spread within the new colony (APENET, 2011). Brood comb removal could be carried out to control swarming behaviour and/or to homogenise the strength of the colonies within the same apiary, leading to a more convenient apiary management by the beekeeper. The quantity of brood in the removed combs should be defined to better define the impact of this removal on the colony.	**H**
	Technical feasibility	Description of management of combs with brood could be provided by a beekeeper through a questionnaire. Management of combs with brood is not included in currently used questionnaires. Comb replacement is already included in currently used questionnaires (NBU questionnaire, 2014; COLOSS questionnaire, 2015).	**H**
	Priority	The use of uncontaminated brood combs is important for ensuring the colony strength, their replacement is a common practice in all the EU Member States as showed in previous studies.	**H**
Replacement of combs with feed sources	Definition	Introduction or removal of food combs (containing pollen and/or honey stores).	
	Variable	Specify quantity of brood, adult bees and feed in the combs managed. 1) Date and number of food combs introduced per colony/year 2) Date and number of food combs removed per colony/year. 3) Number of food comb introduction events per colony/year 4) Number of food comb removal events per colony/year.	

Factors	Criteria	Rationale	Score[a]
	Relevance	Removal or introduction of combs with food (i.e. honey and/or pollen) will decrease or increase the colony strength (consider the strenght definition that also includes the stores), respectively. Introduction is particularly relevant when food resources and/or food stores are low. The quality and quantity of the food introduced influence the nutritional status of the colony. However, the introduction of food combs carries risks related to possible contamination by pesticides (RM Johnson et al., 2010; Mullin et al., 2010) and infectious agents (e.g. AFB, EFB, *Nosema* spp.). Removal of food combs is a practice that could be carried out to homogenise the strength of the colonies within the same apiary, leading to more convenient apiary management by the beekeeper. Also, removed food combs could be reintroduced in periods of dearth. The quantity of food in the introduced/removed combs should be defined to better define the impact of this practice on the colony.	**H**
	Technical feasibility	Description of management of combs with food could be provided by a beekeeper through a questionnaire. Management of combs with food is not included in currently used questionnaires. (Brood) comb replacement is already included in currently used questionnaires (comb by NBU questionnaire, 2014; Brood comb by COLOSS questionnaire, 2015).	**H**
	Priority	The use of uncontaminated brood combs is important for ensuring the colony strength; their replacement is a common practice in all the EU Member States as showed in previous studies.	**H**
Introduction of comb foundation	Definition	Introduction of comb foundation (e.g. combs with a wax sheet/plastic) in the colony.	
	Variable	1) Date and number of comb foundations introduced per colony/year 2) Number of comb foundations introduction events per colony/year.	
	Relevance	The main benefit of the introduction of comb foundation within a colony is that this practice allows the colony to grow in space: bees build the comb from the foundation leading to an increased space for brood rearing/food stores. The timing of the introduction is crucial. Wax could be contaminated by biological (e.g. AFB spores; Ritter, 2003; Pernal et al., 2008) and chemical agents (i.e. miticides; Mullin et al., 2010), therefore good management practices are crucial (i.e. do not reuse AFB-contaminated wax without appropriate sterilisation). However, low risks of biological and chemical contamination are identified because the introduced combs are empty.	**H**
	Technical feasibility	Data related to the introduction of empty combs could be provided by a beekeeper through a questionnaire. The introduction of empty combs is not included in the currently used questionnaires. Comb replacement is addressed in the currently used questionnaires (NBU questionnaire, 2014; COLOSS questionnaire, 2015).	**H**
	Priority	The information gathered on the introduction of empty combs can be used only partially to understand direct effects on the bee health as the risk of contamination is mainly linked to the content of the combs than to their structure.	**L**

Factors	Criteria	Rationale	Score[(a)]
Supplementary feeding	Definition	Supplementary feeding provided to the colony (i.e. sugars, proteins and amino acids, lipids, micronutrients, probiotics inside the hive and water outside the hive).	
	Variable(s)	1) Name of the feeding (e.g. commercial or common name) and physical status (i.e. solid, liquid) 2) Duration [start dd-mm-year, end dd-mm-year if available] and quantity [mg or mL per day/colony] of carbohydrates. (alternatively, ask for kg or litre per colony and make the calculations afterwards) 3) Duration [start dd-mm-year, end dd-mm-year if available] and quantity [mg or mL per day/colony] of proteins (including essential amino acids quantity if known) 4) Duration [start dd-mm-year, end dd-mm-year if available] and quantity [mg or mL per day/colony] of lipids 5) Duration [start dd-mm-year, end dd-mm-year if available] and quantity [mg or mL per day/colony] of water. Other bee feed: 1) Duration [start dd-mm-year, end dd-mm-year if available] and quantity [mg or mL per day/colony] of micronutrients and vitamins (not important see below). 2) Duration [start dd-mm-year, end dd-mm-year if available] and quantity [mg or mL per day/colony] of probiotics (not important see below).	
	Relevance	The quantity of the supplementary feeding influences the nutritional status of the colony and will likely influence its demography. Supplementary feeding may improve colony health enhancing its growth during periods in which nectar/pollen flow is scarce or of low-quality (Schmidt et al., 1995; Mattila and Otis, 2006; Somerville and Nicol, 2006). However, depending on colony demography, time and type of provision, supplementary feeding may be unnecessary and instead have negative consequences on bee health. Supplementary feeding influences bee health in relation to the period it is provided, its nutritional characteristics (e.g. absolute and relative content of amino acids, proteins, sugars, lipids, micronutrients, probiotics) and its content in terms of toxic nutrients, infectious agents/pests and pesticide residues (Barker, 1977a; Schmidt et al., 1995; Mattila and Otis, 2006; Mullin et al., 2010; Pettis et al., 2012; DeGrandi-Hoffman et al., 2010; Di Pasquale et al., 2013). The type and ingredients of the supplemental feeding should be defined (i.e. liquid solution containing sucrose and water, 50% w/w). Supplementary water feeding is provided outside the colony and important in areas where bees have no water available and/or the environmental temperatures are high during certain times of year. Water is necessary for thermoregulation purposes and therefore its availability is related to appropriate brood development.	**H**
	Technical feasibility	Description of supplementary feeding could be provided by a beekeeper through a questionnaire. Supplementary feeding is already included in currently used questionnaires (Brodschneider and Crailsheim, 2010; NBU questionnaire 2014, COLOSS questionnaire 2015). Detailed information on the composition of the supplementary feed could be assessed through laboratory analysis, but this practice is not commonly used by beekeepers because it is expensive. Alternatively, the beekeeper could provide the details from the label of the food supplement, if available.	**H**
	Priority	The use of uncontaminated feed is important for ensuring the colony health, the provision of supplementary feed is a common practice among EU beekeepers (especially professional ones).	**H**

Factors	Criteria	Rationale	Score[(a)]
Production type of the colony	Definition	Product or activity for which the colony is managed.	
	Variable(s)	The colony is managed to produce: 1) honey [Y/N]; 2) pollen [Y/N]; 3) bee packages [Y/N]; 4) royal jelly [Y/N]; 5) queens [Y/N]; 6) nucleus (queen, workers and brood) [Y/N]; 7) propolis [Y/N]; 8) wax [Y/N]; 9) venom [Y/N]; 10) hive rental (pollination service) [Y/N]. Type of production: 1) Protected Geographical Indication (PGI). 2) Protected Designation of Origin (PDO).	
	Relevance	Different practices are used to enhance specific productions, consequently influencing colony demography. Depending on the type of production, colonies typically need to be ready for production in different specific times of the year, i.e. colonies used for honey production need to be ready before the plant species that produce nectar of major commercial interest bloom (i.e. *Robinia*). A specific type of production could impact various other aspects of bee health, i.e. colonies used for pollen production may have a lower amount of pollen stored during certain periods and colonies used for pollination services in specific crops may be highly exposed to pesticides.	**H**
	Technical feasibility	Description of production type of the colony could be provided by a beekeeper through a questionnaire and is already included in a currently used questionnaire (EPILOBEE, 2012–2014). Moreover, the online publication *Agriculture, Forestry and Fishery Statistics* (Eurostat, 2014) gives an overview of the organic farming situation (numbers of beekeepers, tonnes of honey produced) in the EU at the national level.In the EU, 16 categories of honey have been recognised as PDO and three categories as PGI (Evaluation of the CAP Measures Related to Apiculture, Agriculture and Rural Development DG - Final Report, July 2013). Across EU Member States, harmonised statistical data to demonstrate these facts are, however, missing.	**H**
	Priority	The field survey could provide an EU-wide picture of the different production types for better targeting the beekeeping management practices.	**H**

Factors	Criteria	Rationale	Score[(a)]
Change in the number of workers	Definition	Introduction and removal of bees at colony level 'typically in-hive bees'.	
	Variable(s)	1) Addition: 1.1) number of events per colony/year; 1.2) quantity for each event 'weight or number'; 1.3) date of each event; 1.4) origin of the workers for each event 1.4.1) same apiary, 1.4.2) different location (original coordinates). 2) Removal: 2.1) number of events per colony/year; 2.2) quantity for each event 'weight or number'; 2.3) date of each event.	
	Relevance	The introduction of new workers (i.e. package of bees) is beneficial for colony development, increasing colony strength. However, the introduced bees may carry infectious agents, pests and predators (e.g. *Varroa* mites) that could spread within the new colony leading to negative effects on its health. The introduction of new workers influences colony demography (e.g. number of adult bees) and health. The removal of workers (i.e. production of package bees for commercial purposes, insertion of bees in weak colonies) weakens the colony affecting its demography. This is a common beekeeping practice.	**H**
	Technical feasibility	A description of the introduction and removal of new workers could be provided by a beekeeper through a questionnaire. This is not included in currently used questionnaires.	**H**
	Priority	The change in the number of workers is a common practice in all the EU Member States.	**H**
Introduction of a queen bee	Definition	Queen bee introduction into an existing colony.	
	Variable(s)	1) Date of introduction to colony. 2) Reason for introduction: 2.1) original queen bee was intentionally removed (i.e. colony division) [Y/N]; 2.2) original queen bee was missing [Y/N]; 2.3) queen bee improvement [Y/N]. 3) Number of introduction events per colony/year. 4) Introduction success: 4.1) the queen was accepted and successfully laid eggs afterwards; 4.2) rejection rate per colony/year. 5) Geographic origin of the queen bee. 6) Genetic origin of the queen bee (if available) use of molecular markers, such as microsatellites. 7) Produced by the beekeeper within his/her apiary	

Factors	Criteria	Rationale	Score[a]
	Relevance	The introduction of a queen bee is beneficial for colony development in cases where the original queen bee is missing or intentionally removed (e.g. improvement of egg laying rate, age, disease control) and influences various aspects of colony demography (e.g. egg laying rate) and health.	**H**
	Technical feasibility	A description of the introduction of a queen bee could be provided by a beekeeper through a questionnaire. This is already included in a currently used questionnaire (EPILOBEE, 2012–2014). Regarding the identification of the genetic origin of the newly introduced bee queen, it requires the use of laboratory techniques such as molecular markers, that might be expensive in terms of time and money. However, the geographic origin could be provided by the beekeeper through a questionnaire.	**H**
	Priority	The introduction of a queen bee is a common and essential beekeeping management practice and is of EU relevance.	**H**
Migration activity	Definition	Changes in the geographic position of the apiary.	
	Variable(s)	1) Number of migration events per colony/year. 2) Quantity of different apiaries used per colony/year. 3) Date of migration event and distance from previous apiary location; GPS coordinates variation is captured by 'location of the colony' (*x*, *y* coordinates or NUTS region). This information could be derived from the location data of a colony across time (see 'Location of the colony').	
	Relevance	Changing the location of a hive might alter environmental drivers, resource providing unit and the presence/spread of infectious agents, pests and predators, which might affect the health status of the colony. Colony health may also be changed as a consequence of the stressful migratory journey. The number of migratory events and their extent in time and space should be assessed to define the impact of migratory activity on colony health.	**H**
	Technical feasibility	A description of migration activity could be provided by a beekeeper through a questionnaire. This is already included in currently used questionnaires (EPILOBEE, 2012–2014; Van der Zee et al., 2013; NBU questionnaire, 2014; COLOSS questionnaire, 2015).	**H**
	Priority	Migration activity is a common and essential beekeeping management practice and is of EU relevance. In the field surveys it is important to trace back the migration path of the colonies in order to better understand the exposure of the honeybee colonies to the different stressors in different locations.	**H**
Chemical control methods	Definition	Chemical control practices (including veterinary products) are those related to the use of active ingredients (details on dosages, application method and timing are necessary information), such as miticide pesticides (e.g. fluvalinate), organic acids (e.g. oxalic acid) and essential oils (e.g. thymol) to control such as *V. destructor*. Biomechanical control methods are captured by this factor when they are used in combination with an active ingredient.	

Factors	Criteria	Rationale	Score[(a)]
	Variable	Provide for each treatment: 1) Product/active ingredient used [selection from a list of products used in a given Member State]. 2) Target (e.g. *Varroa*) [selection from a list of infectious agents and pests occurring in a given Member State, see Section 3.2.5]. 3) Application method [solid, liquid, gas, other]. 4) Dose/concentration [number (e.g. of strips), mg or mL per colony]. 5) Duration of the treatment [start dd-mm-year, end dd-mm-year].	
	Relevance	Chemical agents used in apiculture act on the infectious agents, pests and/or predators leading consequently to beneficial effects on honeybee health by the reduction of disease impact. On the other hand, they could elicit adverse side effects on honeybees. In fact, chemical treatments are typically applied inside the hive and those molecules are, therefore, often found as residues in various bee matrices such as wax, bee bread and honey (Mullin et al., 2010; Boncristiani et al., 2012; RM Johnson et al., 2013). Chemical agents are used by many beekeepers throughout Europe (e.g. acaricides for *Varroa* control).	**H**
	Technical feasibility	Description of chemical control method used, including the active agents, dosage and timing could be provided by a beekeeper.This is already included in currently used questionnaires (Brodschneider and Crailsheim, 2010; EPILOBEE, 2012–2014; Van der Zee et al., 2013; NBU questionnaire, 2014; COLOSS questionnaire, 2015).	**H**
	Priority	Chemical treatments in apiculture are of EU relevance and documented have been previous projects (see above). Some chemical treatments are recognised as major stressor influencing the health of the honeybee colonies throughout the world e.g. miticides against Varroa mite (Aumeier and Ziegelmann, 2010; Mullin et al., 2010; Rosenkranz et al., 2010).	**H**
Physical/ mechanical control methods	Definition	Physical/mechanical control methods used (including timing) and respective target. They include the use of heat, cold, light, humidity, carbon dioxide, light, ventilation or sound to control a pest.	
	Variable(s)	1) Number of physical control events/year. 2) *Varroa* control methods with no chemical use associated: 2.1) worker brood removal [Y/N]; 2.2) drone brood removal [Y/N]; 2.3) queen confinement [Y/N]; 2.4) temperature (heat/cold) treatment of brood/bees [Y/N]; 2.5) mesh board [Y/N]. 3) Shook swarming (i.e. AFB) [Y/N]. 4) Trapping effort: total number of days of trapping $\sum_i (Ti \times t)$ (trapping effort): 4.1) Small hive beetle at colony level *Ti*; 4.2) *Vespa velutina*: at apiary level *Tj*; 4.3) Other *Vespa* (i.e. *V. orientalis*): at apiary level *Tk*.	

Factors	Criteria	Rationale	Score[(a)]
		5) Other methods for Vespa spp. (addressed in simplified manner through questionnaire) 6) Sterilisation of Non-living contaminated material by: 6.1) gamma-rays [Y/N]; 6.2) heat treatment by flames. Y/N; 6.3) no sterilisation, but disposal [Y/N]; 6.4) neither sterilisation nor disposal [Y/N]; 6.5) steam; 6.6) other treatments. 7) Storage of beekeeping materials, including supers and frames: 7.1) closed and uncontaminated facilities [Y/N]; 7.2) refrigerated facilities [Y/N]; 7.3) controlled atmosphere facilities – CO_2 treatment [Y/N].	
	Relevance	Physical methods used in apiculture act on the infectious agents/pests/predators leading to typically positive effects on honeybee health. However, inappropriate physical control methodologies could have adverse side effects on honeybee health, e.g. inefficient sterilisation of contaminated non-living material (i.e. hives containing AFB spores) may lead to further contamination later in time, if the material is reused. Various physical control methods are used by many beekeepers throughout Europe, e.g. hive sterilisation, traps for bee predators (Neumann and Hoffmann, 2008).	**H**
	Technical feasibility	Description of the physical control method used, including timing could be provided by a beekeeper. This is included in a currently used questionnaire (COLOSS questionnaire, 2015).	**H**
	Priority	The use of physical methods is highly variable depending on the type and area of production in EU Member States.	**M**
Biological control methods	Definition	Biological control methods used (including agents, dosages and timing) and respective target.	
	Variable	Dose/type of agent/method used/frequency or duration.	
	Relevance	Biological control methods used in apiculture act on the infectious agents, pests and/or predators leading consequently to beneficial effects on honeybee health. However, they could elicit adverse side effects on honeybee health. Biological agents are rarely used by beekeepers throughout Europe.	**L**
	Technical feasibility	Description of biological control method used, including the agents, dosage and timing could be provided by a beekeeper. This is not included in currently used questionnaires. No assessment done because the evidence that this factor is linked with bee health is low.	**NA**
	Priority	Not assessed.	**NA**

Factors	Criteria	Rationale	Score[(a)]
Swarm control	Definition	Beekeeping practices used to control the swarming behaviour of the colony.	
	Variable(s)	1) Number of interventions per colony/year (swarm control effort). 2) Queen cell removal: 2.1) date of first event 2.2) number of cells per colony/year. 3) Management of colony size: 3.1) addition of empty frames (i.e. wax sheets) [Y/N] 3.2) removal of brood and adult bees [Y/N]. NOTE: Date (period) of swarming fever could be considered as a TIME 0 to compare the development (etc.) of the colony between geographical regions and climates across Europe.	
	Relevance	Swarm control practices influence the demography of the colony and are therefore relevant for the interpretation of other parameters related to bee health. The specific methodology used should be defined.For example, Mutinelli et al. (2014) described the use of artificial swarms (moving part of the colony population to another hive) to prevent natural swarming and to reduce possible pest dispersal in small hive beetle affected areas.	**H**
	Technical feasibility	A description of the swarm control method could be provided by a beekeeper through a questionnaire. This is not included in currently used questionnaires.	**H**
	Priority	Data collected in field surveys on swarm control practices cannot be used directly in assessing the effects on the health of honeybee colonies, and need to be interpreted in term of effects on the honeybee colony demography. Although data could be provided through questionnaires, specific beekeeper skills are needed to accomplish swarm control practices successfully across EU, and because of the difficulty in standardising these practices, this factor is scored medium.	**M**
Apiary characteristic and management			
Location of the apiary	Definition	The location of the apiary and colony	
	Variable(s)	1) Location of the colony 2) [*x*, *y* coordinates] at date [dd-mm-year] 3) Location of the apiary (centroid or NUTS region) 4) Spatial distribution of the colonies within the apiary: 4.1) average distance between the colonies of the same apiary.	
	Relevance	The location of the apiary is related to its health, being linked with environmental drivers and the RPU.	**H**

Factors	Criteria	Rationale	Score[a]
	Technical feasibility	The location of the apiary could be provided by a beekeeper through a questionnaire. This is already included in currently used questionnaires (EPILOBEE, 2012–2014; US National Honey Bee Disease and Pest Survey questionnaire, BeeNet, 2013; NBU questionnaire, 2014; COLOSS questionnaire, 2015). Some Member States have systems in place to register apiary locations (e.g. France, Italy). However accessibility to these data is still to be assessed.	**H**
	Priority	Same reasoning as Migration activity above.	**H**
Size of apiary	Definition	Number of colonies composing the beekeeper's apiary.	
	Variable	1) Number of colonies. 1.1) Total number of colonies 1.1.1) Number of productive colonies (i.e. colonies that are used by the beekeeper for production, and successfully yield a production). 1.1.2) Change in number of colonies. 1.1.2.1) Addition. 1.1.2.1.1) Number of colonies added. 1.1.2.1.2) Date of the event. 1.1.2.1.3) Origin of the colony. 1.1.2.1.3.1) Same apiary. 1.1.2.1.4) Artificial swarm [Y/N]. 1.1.2.1.5) Natural swarm [Y/N]. 1.1.2.1.5.1) Different location. 1.1.2.1.5.1.1) Original GPS coordinates of the introduced colony. 1.1.2.2) Removal (i.e. 'merge' if colonies are from the same apiary). 1.1.2.2.1) Number of colonies removed. 1.1.2.2.2) Date of the event. 1.1.2.2.3) Removal rate per apiary/year. 1.1.2.3) Dead colonies 1.1.2.3.1) Date of colony death. 1.1.2.3.2) Number of dead colonies per year.	

Factors	Criteria	Rationale	Score[a]
	Relevance	Apiary size (including the addition of colonies from other apiaries) influences the risk of dissemination of infectious agents, pests and predators and plays a role in the resource providing unit availability.	**H**
	Technical feasibility	A description of the introduction of colonies could be provided by a beekeeper through a questionnaire. This is already included in currently used questionnaires (EPILOBEE, 2012–2014; NBU questionnaire, 2014).	**H**
	Priority	The size of apiaries is very variable depending on their production goals and their geographical location in EU Member States.	**M**
Proximity of colonies belonging to other apiaries	Definition	Interaction between bees from colonies in the same resource providing unit.	
	Variable(s)	1) Number of hives located within the double the foraging distance (2–5.5 km) and the surface of the shared foraging area.	
	Relevance to understand the context	Vicinity of colonies presenct within double the maximum foraging distance may increase the risk of dissemination of infectious agents, pests and predators and plays a role in the resource providing unit availability, i.e. low food availability/colony if high colony density in an area with low food resources. EFSA (2012a) indicates the median, mean and max foraging distances reported by: Visscher and Seeley (1982) (median, 1.6 km; mean, 2.2 km; max, 10.9 km); Beekman and Ratnieks (2000) (median, 6.1 km; mean, 5.5 km; max, 12 km); Steffan-Dewenter and Kuhn (2003) (median, 1.2 km; mean, 1.5 km; max, 10 km). The panel suggests using an average foraging distance of 3 km around the colony.	**H**
	Technical feasibility	This information could be included in a questionnaire, although accurate data regarding the proximity of other apiaries may not be easily available to individual beekeepers. Some Member States have systems in place to register apiary locations (e.g. France, Italy). However accessibility to these data is still to be assessed.	**L**
	Priority	Not assessed.	**NA**

(a): The scores are explained in Table 3, Section 2.2.1. The score H-HH is highlighted in green as the factors with these score are taken forward in TOR3, whereas the other factors are not.

Table B.9: Analysis of factors related to colony outputs

Factor (definition)	Criteria	Rationale	Score[(a)]
Provisioning service	Definition	The amount and quality of the outputs represent the service provided by the bee colony to the ecosystem. Millennium Ecosystem Assessment (2005) defines the ecosystem services from an anthropocentric perspective and considers ecosystem provision when humans benefit from the environment. In this context, the harvested honeybee products, the extraction of live bees from the hive and the use of the hive to provide pollination to specific crops against remuneration of the beekeeper correspond to an ecosystem provisioning service.	
	Variables	Harvested quantity of: 1) honey 2) Pollen 3) propolis 4) wax 5) jelly 6) venom. Number of live honeybees extracted from the hive: 1) queens 2) nucleus (start-up colonies). Hive rental service (partly covered under section on Beekeeping management practices and migratory activity): 1) income (% of income) 2) frequency.	
	Relevance	An analysis of the outputs can provide information on overall bee colony performance and therefore, an assessment of the health status of a managed honeybee colony should include measurement of the outputs in terms of provisioning service – for the harvested products, the hive rental service and the live honeybees.	**H**
	Technical feasibility	Weighing the super is the most frequently used method, whereas digital image analysis and visual estimation are also possible. More details are provided in Table C.32	**H**
	Priority	This factor is of EU relevance.	**H**
Regulating service (pollination service)	Definition	The pollination service provided by the bees corresponds to an ecosystem-regulating service. It needs to be assessed for both the crops and the wild plants that benefit from the pollination. Pollination demand is defined as the spatial and temporal pattern of flowers requiring pollination and the pollination supply is defined as the spatial and temporal pattern of successfully pollinated flowers.	
	Variables	1) Supply (flowers of crops/wild plants): 1.1) crop yield (tonnes/ha) 1.2) plant fitness (viable seeds/optimal crop produced) 1.3) Pollen transfer (number of pollen grains deposited) 1.4) Pollinator visitation (visits to flowers).	

Factor (definition)	Criteria	Rationale	Score[(a)]
		2) Demand (pollinated flowers of crops/wild plants): 2.1) dates of flowering periods in the RPU 2.2) number of flowers (month/season) 2.3) phenological stage of the plants (receptive/non-receptive)	
	Relevance	Honeybees visit a large number of plants, and will provide a substantial proportion of pollination services to many of them (Delaplane and Mayer, 2000). Measurements of pollination services to multiple plants within the wider landscape therefore represents a tangible measure of overall colony health as weak colonies in poor health will provide little pollination services or only provide services to the most beneficial resources	**H**
	Technical feasibility	The time and expertise demanded by the field work involved in most viable methods to evaluate supply and demand make this a low feasibility indicator for most methods However, if sufficient data are available on hive locations, modelling approaches are highly viable. There is a significant lack of information that quantitatively links pollination services to colony health; however, using modelling approaches it is possible to link this with other colony attributes and external drivers (see Table C.33 for methods for measuring pollination demand and supply)	**H**
	Priority	The provision of pollination service is assessed as medium priority considering that: it is not realistic to retrieve data for the above mentioned variables in the context of EU wide field survey addressed to beekeepers; and the variability of honeybee habitats in the EU	**M**

(a): The scores are explained in Table 3, Section 2.2.1. The score H-HH is highlighted in green as the factors with these score are taken forward in TOR3, whereas the other factors not.

Appendix C – Measurement of selected indicators and factors

C.1. Colony attributes

C.1.1. Queen presence and performance

Table C.1: Visual verification of queen's presence by checking through combs

Indicator		**Assessing variable [unit]**	**Test method**
Presence of a queen		Presence of a queen [Y/N]	Visual verification of queen's presence by checking through combs
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	To determine whether queen is present and alive. This is easier to perform when the queen is labelled (Human et al., 2013). If the queen is not seen by the beekeeper, this suggests that she may not be there. However, it may also be that she was just not spotted or she lost her tag, in which case the beekeeper will then check for the presence of 1-day-old eggs	
	Sampling (matrix, number, temporal and spatial aspects)	Inspect all hive compartments until the queen is seen (it is not possible to see the queen during the queen succession period). This is to be done every time the hive is open (or, as a minimum, during spring/summer/ autumn routine checks)	
Evaluation	Test sensitivity and LOD/LOQ	Test sensitivity is assumed to be high, especially if the queen is labelled (Human et al., 2013). However, if she is not labelled, it is sometimes challenging to see the queen during a routine check. Therefore, if the queen is unlabelled, there is a possibility of a false negative. A beekeeper will then look for the presence of 1-day-old eggs as an indirect way to know if the queen is present. LOD/LOQ is not known	
	Test specificity	Test specificity is assumed to be high. The queen is morphologically and behaviourally distinct (Winston, 1991), so it is unlikely that a worker or a drone will be incorrectly classified as a queen	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	Checking for the queen is done routinely and quickly, usually in under 5 min per hive, by a beekeeper. The method is used by a large majority of hobby and commercial beekeepers throughout the EU. Only standard beekeeping equipment for opening hives (veil, smoker, hive tool) is necessary	
	Standardisation	If the queen is labelled, the method is easily applicable. The method can still be applied if the queen is not labelled, although there may be an increased chance of a false negative (which can be reduced by checking for 1-day-old eggs)	
	Possible limitations to use in field survey	No practical limitations	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.2: Visual identification of the presence of eggs, larvae, and pupae

Indicator		**Assessing variable [unit]**	**Test method**
Potential fecundity		Presence of viable worker brood (eggs, larvae and pupae) [Y/N]	Visual identification of the presence of eggs, larvae and pupae
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	To determine if the queen is laying viable eggs and if the colony is able to rear the eggs to adulthood	
	Sampling (matrix, number, temporal and spatial aspects)	Check the hive (comb surface) until the presence of the three life stages is verified. Should be done every time the hive is open (minimum during spring, summer, and autumn routine checks). No brood will be reared during early winter or during extreme weather events. Egg-laying will also cease immediately prior to swarming	
Evaluation	Test sensitivity and LOD/LOQ	Test sensitivity is assumed to be high. If brood is not present in all three stages during a season when it should be there, this is an indication that the hive is not healthy. Additionally, all three stages of brood are a highly visible and easily recognisable feature (Winston, 1991). There is very little chance of a false negative. LOD/LOQ is not known	
	Test specificity	Test specificity is assumed to be high. If the queen is no longer laying fertilised eggs, the brood will all be drone brood, which is irregular looking and easy to recognise. Therefore, there is little chance of a false positive	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	Checking for all three stages of brood is done routinely and quickly, usually in just a few minutes, by a beekeeper. The method is used by a large majority of hobby and commercial beekeepers throughout the EU. Only standard beekeeping equipment for opening hives (veil, smoker, hive tool) is necessary	
	Standardisation	The method is easily applied and implemented during routine checks	
	Possible limitations to use in field survey	No practical limitations	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.3: Record keeping and queen marking

Indicator		**Assessing variable [unit]**	**Test method**
Queen longevity		Age of queen in months [number]	Record keeping and queen marking either with a number tag or using a colour code
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	To determine the age of the queen through marking and record keeping or through systematic labelling using a colour code (international code of colour; Human et al., 2013)	
	Sampling (matrix, number, temporal and spatial aspects)	Every time the hive is inspected, it should be assessed whether the queen identified in the last visit is still present or whether she has been replaced by a new queen	
Evaluation	Test sensitivity and LOD/LOQ	Test sensitivity is assumed to be high and low, depending on whether the queen was labelled or not, respectively, given that the beekeeper implements record keeping of the queens per hive	
	Test specificity	Test specificity is assumed to be high and low, depending on whether the queen was labelled or not, respectively	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	Can only be determined by accurate marking, either with a number tags, which are available from all honeybee suppliers, in conjunction with record keeping; or with paint pens, also easily available from all honeybee suppliers, and in accordance with international colour code. Marking the queen is practised widely by hobby and commercial beekeepers throughout the EU and takes only a few minutes once a new, unlabelled queen is found	
	Standardisation	Using international code colours can facilitate the process of determining queen age (Human et al., 2013). Otherwise the beekeeper can rely on his/her record keeping	
	Possible limitations to use in field survey	No practical limitations. However, if beekeeper regularly replaces queen (e.g. every 6 months), regardless of her performance and observable fecundity, then that should be noted, and this variable becomes less meaningful	

LOD: limit of detection; LOQ: Limit of quantification.

C.1.2. Demography

Table C.4: Visual estimation of the number of combs covered by adult bees

Indicator		**Assessing variable [unit]**	**Test method**
Colony size		Number and type of combs covered by adult bees [number of combs], [type of comb]	Visual estimation of the number of combs covered by adult bees
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	Determining the proportion of comb surface covered by adult bees by visual estimation and reporting it as total number of combs fully covered by in each hive (i.e. 3.5 combs, 6 combs, etc.). It is recommended to gently remove the combs from the hive to ensure that the bees stand in the comb surface. Estimation of comb coverage should then be carried out sequentially for both sides of each frame (Costa et al., 2012). For subsequent determination of the total number of adult bees (i.e. during the data analysis step), it is also necessary to consider the frame type, which should be reported. Estimating the average number of bees in the comb increases the precision of the calculation (because this varies according to the bee subspecies), however, if this value is not available, data from literature can be used (e.g. table 2 of Delaplane, 2013)	
	Sampling (matrix, number, temporal and spatial aspects)	Both sides of all frames containing adult bees should be inspected. Estimation of the colony size should be performed before estimation of the number of brood cells. The assessment should be done at least three times a year: after wintering, during summer and before wintering	
Evaluation	Test sensitivity and LOD/LOQ	The sensitivity of this method depends on the observer's ability to accurately estimate the percentage of comb surface fully covered by adult bees. Because the number of bees on combs will vary according to time of day and bee foraging activity, the results may slightly underestimate the real colony size. To obtain more accurate results, calculation of the total number of bees should be done when most bees, if not the whole population, is present in the colony, for example, by closing hive entrances in the early morning until bees are counted (Delaplane et al., 2013a,b).	
	Test specificity	Test specificity is assumed to be high because the adult bees are easily recognised by visual observation.	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	Every beekeeper that has had a few lessons on beekeeping should be able to implement this method. A couple of minutes per hive are sufficient to perform this assessment. It is to be expected that the accuracy of this method is higher when the adult bees are massed together in convenient contiguous patches. Only standard beekeeping equipment for opening hives (veil, smoker, hive tool) is necessary. Assessment of the average number of bees per cm^2 is likely to take more time than the visual observation of the comb coverage by the adult bees, but as explained above, data from the literature can be used. Probably, only a few beekeepers implement this method at the moment, but most of them will be able to do it	
	Standardisation	The method is not standardised and is used in an informal way by beekeepers. It is assumed that the results will have less variability if beekeepers undergo short training to learn how to apply the method. It is recommended that the method is performed simultaneously by two observers, who independently score the percentage of comb covered, to reduce variation between observations. Ideally, each observer is accompanied by a dedicated secretary who writes down numbers, or each observer fitted with an audio-recorder (Delaplane 2013)	
	Possible limitations to use in field survey	No practical limitations were identified	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.5: Weighing the worker population and calculating the number of bees considering an average weight of individuals

Indicator		**Assessing variable [unit]**	**Test method**
Colony size		Total number of adult bees derived from population weight [number of adult bees], [type of comb]	Weighing the worker population and calculate the number of bees considering an average weight of individuals
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	The whole hive is weighed, then opened, all are bees brushed off every comb and surface (usually into a temporary holding hive), and the hive is reweighed without bees. The difference in weight equals the net weight of bees. By knowing the weight of all the workers in a colony, it is easy to deduce their total number. For data analysis purposes, it is generally considered that the average weight of one worker bee is 100 mg (Delaplane et al., 2013a,b). For more precision in calculating the total number of worker bees in a colony, it is also possible to determine the average weight of the workers. A sample of around 300 live bees can be collected into a preweighed container, the bees frozen or narcotised with cold or CO_2, and counted to determine average fresh weight (mg) per bee. Net colony bee weight can then be divided by average fresh weight per bee to derive colony bee population (Delaplane et al., 2013a,b)	
	Sampling (matrix, number, temporal and spatial aspects)	The sampling matrix is the hive, with and without adult bees. Estimation of the colony size should be performed before the estimation of the number of brood cells (see Appendix F). The assessment should be done at least three times a year: after wintering, summer and before wintering	
Evaluation	Test sensitivity and LOD/LOQ	The sensitivity is reported to be high (Delaplane et al., 2013a,b). The results may slightly underestimate the real colony size because when the hive is opened a certain number of bees fly and will not be counted. Also, this method does not take into account the foragers that had left the colony at the time of measurement. To obtain more accurate results, the calculation of total number of bees should be done when most bees, if not the whole population, is present in the colony (Delaplane et al., 2013a,b)	
	Test specificity	Test specificity is assumed to be high	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	It is necessary to use a scale with sufficient accuracy. Presentation of the weight in the form of a number (digital display) is preferred over a needle display. The method can be applied by any beekeeper and does not require training other than in manipulating bees. Currently, the method is mainly applied by researchers	
	Standardisation	The method is not standardised and is used in an informal method used by beekeepers. The method is described, for instance, by Delaplane et al. (2013a,b). Alternative approaches might be available to determine the net weight of a worker population, but it is assumed they will lead to a comparable result, given that an accurate scale is used	
	Possible limitations to use in field survey	An accurate scale is needed	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.6: Visual estimation of the comb surface covered by brood cells

Indicator		**Assessing variable [unit]**	**Test method**
Brood		Number of combs covered by brood cells [number of combs], [type of comb]	Visual estimation of the comb surface covered by brood cells
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	Estimating the proportion of comb surface covered by brood cells and reporting it as number of combs fully covered by brood in each hive (e.g. 2.5 combs, 6 combs, etc.). Estimation of comb coverage should be carried out sequentially for both sides of each frame (Costa et al., 2012). For subsequent determination of the number of brood cells in the hive (i.e. during the data analysis step), it is also necessary to consider the comb type, which should also be reported. Estimating the density of brood cells in the comb increases the precision of the calculation, however, if this value is not available, data from literature can be used to estimate the total number of bees (table 2 in Delaplane 2013). For example, for a 'Dadant'-type comb in Europe, the surface area of the side of a comb is 1,130 cm^2, and the number of brood cells per cm^2 is 4. During this estimation it is easy to observe the quality of the brood and especially the brood pattern consistency in order to estimate whether it is a 'spotty' brood (e.g. > 10% of empty cells)	
	Sampling (matrix, number, temporal and spatial aspects)	Both sides of all combs containing brood should be inspected. Brood quantification should be done at least three times a year: after wintering, during the peak of colony activity and before wintering	
Evaluation	Test sensitivity and LOD/LOQ	It is assumed that this method has low/medium sensitivity because it can be difficult to observe and quantify small variations in brood surface. Furthermore, the sensitivity of this method depends on the observer's ability of correctly estimate the comb surface covered by brood	
	Test specificity	Test specificity is assumed to be high because it is easy to distinguish brood from other substrates by visual observation	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	A couple of minutes is sufficient to perform this assessment per hive This method it is quick and can be implemented by any beekeeper. No training other than managing bees is required and only standard beekeeping equipment for opening hives (veil, smoker, hive tool) is necessary	
	Standardisation	The method is not standardised and is used in an informal way by beekeepers. The method is described, for instance, by Delaplane et al. (2013a,b)	
	Possible limitations to use in field survey	No practical limitations were identified	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.7: Estimation of the comb surface covered by brood cells using digital photography and image analysis

Indicator		**Assessing variable [unit]**	**Test method**
Brood		Total comb surface covered by brood cells determined by digital photography and image analysis [cm^2]	Estimation of the number of brood cells using digital photography and image analysis
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	Determination of the comb surface covered by brood cells by taking a digital photograph of each side of a comb containing brood, followed by image analysis. The comb surface can be transformed into the number of individuals using indexes specific to the subspecies or derived considering a sample of cells and measuring its surface (Delaplane et al., 2013a,b). This method allows the number of different types of brood (eggs, larvae, pupae) to be determined more easily than in the field by visual observation. During this estimation it is easy to observe the quality of the brood and especially the brood pattern consistency in order to estimate whether it is a 'spotty' brood (e.g. > 10% empty cells)	
	Sampling (matrix, number, temporal and spatial aspects)	Both sides of all combs containing brood should be sampled. The assessment should be done at least three times a year: after wintering, during the beekeeping season or before wintering	
Evaluation	Test sensitivity and LOD/LOQ	Test sensitivity is assumed to be high due to the high sensitivity of image analysis using appropriate software	
	Test specificity	Test specificity is assumed to be high due to the high capacity of image analysis using appropriate software to discriminate brood from other matrices in the hive	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	Besides standard beekeeping equipment to open the hive, access to a digital camera and a computer with image analysis software is required. It is likely that applying this method takes more time than visually estimating the number of brood because it is necessary to fully remove the adult bees from the comb frame and to take a picture of each side of the comb. Analysing the pictures afterwards can be time consuming and requires knowledge of an image analysis programme. There is software freely available on internet. This method is mainly used for research purposes	
	Standardisation	The method is described, for instance, by Delaplane et al. (2013a,b). Results obtained using different software packages are considered to be comparable	
	Possible limitations to use in field survey	Practical limitations hamper the use of this method when many hives have to be analysed. The image analysis can be time consuming. However, the advantage is that there is the possibility to go back to the stored images, and use them for comparisons over time or exchange the with other observers for comparison in space	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.8: Estimation of the comb surface covered by brood cells using a grid

Indicator		**Assessing variable [unit]**	**Test method**
Brood		Total comb surface covered by brood cells [cm^2]	Estimation of the comb surface covered by brood cells using a grid
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	Determining the area of comb surface covered by brood cells using a transparent plastic grid premarked in cm^2 and visually summing the area of brood (Delaplane et al., 2013a,b). The brood area estimation should be carried out sequentially for both sides of each comb. For subsequent determination of the total number of brood cells (i.e. during the data analysis step), the total area can be multiplied by the average number of cells per cm^2 Estimating the average number of cells per cm^2 in the comb increases the precision of the calculation, however, if this value is not available, data from the literature can be used (table 2 in Delaplane et al., 2013a,b). During this estimation, it is easy to observe the quality of the brood and especially the brood pattern consistency in order to estimate whether it is a 'spotty' brood (e.g. > 10% empty cells)	
	Sampling (matrix, number, temporal and spatial aspects)	Both sides of all combs containing brood should be sampled. The assessment should be done at least three times a year: after wintering, during the beekeeping season or before wintering	
Evaluation	Test sensitivity and LOD/LOQ	The sensitivity of this method is assumed to be medium because the use of grids should help to observe and quantify small variations in brood surface	
	Test specificity	The specificity of this method is assumed to be high because it is easy to distinguish brood from other substrates by visual observation	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	Besides standard beekeeping equipment for opening hives (veil, smoker, hive tool), a transparent grid with graduations is necessary. It is expected that the accuracy of this method is best when the brood cells are massed together in convenient contiguous patches. The most difficult aspect is to sum all the areas of brood. It is likely that this method takes a couple of minutes per comb to be performed. The area calculations can be done at a later stage and it is assumed that the time needed is negligible (simple multiplication). The method is probably used only rarely at the moment	
	Standardisation	The method is not standardised. The method is described, for instance, by Delaplane et al. (2013a,b). It is assumed that the results will have less variability if beekeepers undergo short training to learn how to apply the method	
	Possible limitations to use in field survey	No practical limitations	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.9: Estimation of the comb surface covered by brood cells using ellipse surface area measurement

Indicator		**Assessing variable [unit]**	**Test method**
Brood		Total comb surface covered by brood cells [cm^2]	Estimation of the comb surface covered by brood cells using ellipse surface area measurement
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	Brood surfaces, on both side of each brood frame, are assimilated to ellipses (Fresnaye, 1961; Mallet and Charles, 2001; Vallon et al., 2008). Therefore, the surface of the brood could be approximated by measuring the length (L; cm) and width (W; cm) of the brood then using the formula: surface (S; cm^2) = ($L \times W \times \Pi$). Brood surfaces are then totalled across all the combs of each colony. For the subsequent determination of the number of brood cells, the surface (cm^2) of brood is multiplied by the average number of cells per cm^2 (Odoux, 2014). Estimating the average number of cells per cm^2 in the comb increases the precision of the calculation, however, if this value is not available, data from the literature can be used (table 2 in Delaplane et al., 2013a,b). During this estimation it is easy to observe the quality of the brood and especially the brood pattern consistency in order to estimate whether it is a 'spotty' brood (e.g. > 10% empty cells)	
	Sampling (matrix, number, temporal and spatial aspects)	Both sides of all combs inside the hive should be sampled. The assessment should be done at least three times a year: after wintering, during the beekeeping season and before wintering.	
Evaluation	Test sensitivity and LOD/LOQ	The sensitivity of this method is assumed to be medium because it is related to the ability of the observer to properly measure the dimensions of ellipses	
	Test specificity	It is assumed that this method has a high specificity because it is easy to distinguish brood from other substrates	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	Besides standard beekeeping equipment for opening hives (veil, smoker, hive tool), a ruler is required. It is expected that the accuracy of this method is best when the brood cells are massed together in convenient contiguous patches. It is likely that this method takes a couple of minutes per comb to be performed. The area calculations can be done in a later stage and it is assumed that the time needed is negligible (simple multiplication). The method is probably used only rarely at the moment	
	Standardisation	The method is not standardised. It is described by Odoux et al. (2014). It is assumed that the results will have less variability if beekeepers undergo short training to learn how to apply the method	
	Possible limitations to use in field survey	No practical limitations were identified	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.10: Visual estimation of the percentage of brood comb surface covered by nurses

Indicator		**Assessing variable [unit]**	**Test method**
Nurses		Brood comb surface covered by adult bees [percentage]	Visual estimation of the percentage of brood comb surface covered by nurses
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	It is important to check whether the open brood has enough nurses to take care of it (particularly to feed the larvae), and whether all the brood (including pupae in the closed cells) has enough bees to warm it (in relation to the temperature of the environment). When the observer estimates visually the surface of the brood (see Brood Demography), each side of a brood comb also has to be assessed to determine whether the entire surface of the brood is covered by nurses. If it is the case, it means that the coverage is 100%. If it not the case, the observer should visually estimate the surface of the brood covered by nurses, for each brood comb. The observer then has to estimate the total percentage coverage by the nurses of all the brood combs of a colony	
	Sampling (matrix, number, temporal and spatial aspects)	Both sides of all combs containing brood should be sampled. The assessment should be done at least three times a year: after wintering, during the beekeeping season and before wintering. It should be performed at the same moment of brood estimation	
Evaluation	Test sensitivity and LOD/LOQ	It is assumed that this method has a low/medium sensitivity. The sensitivity of this method depends on the observer's ability to correctly estimate the brood surface covered by nurses	
	Test specificity	The specificity is assumed to be low because bees performing tasks other than nursing building combs, cleaning, etc.) may also be on the combs and be erroneously be classified as nurses	
	Required time, equipment, expertise and availability/ dissemination of method (sampling/analysis)	Only standard beekeeping equipment for opening hives (veil, smoker, hive tool) is necessary. For more accurate results, it is recommended that this method is performed simultaneously by two observers, who independently score the percentage of brood covered by adult bees. Preferably, each observer is accompanied by a dedicated secretary who writes down the numbers, or the observer uses an audio recorder (Delaplane et al., 2013a,b). An average result is then calculated and the final results reported. A couple of minutes is sufficient to perform this estimation. It is expected that the accuracy of this method is higher when the nurses are massed together in convenient contiguous patches. Beekeepers roughly check if brood is covered more or less by nurses, but they do not quantify the surface or number of nurses	
	Standardisation	The method is not standardised. Assessing the number of adult bees by visual observation is described by Delaplane et al. (2013a,b), but estimation of the number of nurses is not. This method was adapted slightly as an option for the assessment of the number of nurses	
	Possible limitations to use in field survey	No practical limitations were identified	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.11: Visual estimation of the number of dead bees in the vicinity of the hive, in the hive entrance and in the bottom of the hive

Indicator		**Assessing variables [unit]**	**Test method**
Dead bees		1) Number of dead bees in the vicinity of the hive 2) Number of dead bees in front of the hive (in the flight board) 3) Number of dead bees inside the hive (in the bottom of the hive)	Visual estimation
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	Estimating by visual observation the approximate number of dead bees in the vicinity of the hive, in front of the hive and inside the hive	
	Sampling (matrix, number, temporal and spatial aspects)	The area in the vicinity of the hive (~ 2 m around the hive) and the area in front of the hive (in the flight board) should be inspected and the number of dead bees estimated. The hive bottom should also be inspected after removing the combs	
Evaluation	Test sensitivity and LOD/LOQ	The sensitivity of estimating the number of dead bees in the vicinity of the hive by visual observation will vary according to the environment surrounding it (vegetation might impair the detection of dead bees, for example). Foragers usually die in the foraging area and consequently it is not expected that a high number of bees will be found in the hive surroundings under normal conditions. Moreover, dead bees around the hive are often dispersed by wind or eaten by predators (wasps, etc.), hence the number of dead bees can be greatly underestimated. For these reasons, this method is considered to have low sensitivity, however, if a large number of dead bees is present, the beekeeper will be able to detect it. The sensitivity of visually inspecting the hive bottom for the presence of dead bees is also considered to be medium/high because under normal conditions dead bees inside the hive are removed by the workers. The presence of dead bees inside the hive may be indicative of a health problem	
	Test specificity	It is assumed that this method has high specificity because it is easy to distinguish dead bees	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	Careful inspection of the hive surroundings and the bottom of the hive might take ~ 2–3 min. No special equipment is needed	
	Standardisation	Visual inspection of the hive surroundings, of the flight board and of the hive bottom to check for the presence of dead bees is already performed by beekeepers	
	Possible limitations to use in field survey	No limitations were identified	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.12: Visual estimation of brood pattern consistency

Indicator		**Assessing variables [unit]**	**Test method**
Brood pattern consistency		Estimating percentage of empty brood cells in a 10 × 10 area [%]	Visual estimation
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	Visual observation of the brood pattern consistency (qualitative variable) A 'spotty' brood (> 10% empty cells) can be sign of a problem related to sperm quality, or be indicative of the presence of infectious agents or pests (e.g. *Varroa*)	
	Sampling (matrix, number, temporal and spatial aspects)	Brood pattern consistency is determined by placing a grid that delimits 100 cells over a section of the sealed brood and subtracting empty cells to estimate percentage brood solidness. This measure is repeated on different patches of brood to derive a mean of at least 10 observations	
Evaluation	Test sensitivity and LOD/LOQ	The sensitivity could be quite high if the 10 observations are made at random on the brood combs	
	Test specificity	The test is considered specific because it is easy to distinguish brood combs	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	The equipment is very simple, a piece of cardboard with a square equal in size to 10 × 10 cells is laid over a patch of brood. Percentage brood solidness is measured directly as (100 – no. empty cells) and can be estimated at the same time as evaluation of the brood surface	
	Standardisation	The method is not standardised, but used currently in field studies (Delaplane et al., 2013a,b)	
	Possible limitations to use in field survey	No limitations were identified	

LOD: limit of detection; LOQ: Limit of quantification.

C.1.3. In-hive products

Table C.13: Visual estimation of the comb surface covered by bee bread

Indicator		**Assessing variable [unit]**	**Test method**
Quantity of bee bread		Number of combs covered by bee bread [number of combs], [type of comb]	Visual estimation of the comb surface covered by bee bread
Test characteristics		**Information on the method of analysis**	
Protocol	Test protocol	As detailed in Table C.6. Estimating the density of bee bread cells in the comb increases the precision of the calculation, however, if this value is not available, data from the literature can be used to estimate bee bread quantity (table 2 in Delaplane et al., 2013a,b)	
	Sampling (matrix, temporal and spatial aspects)	Both sides of all frames containing bee bread should be inspected. Bee bread quantification should be done at least three times a year: after wintering, during the beekeeping season and before wintering (see Section 2.2.2). The period of prewintering is crucial because the quantity of bee bread is very important for successful resumption of the development and activity of the colony after the wintering period	
Evaluation		Identical to Table C.6	

Table C.14: Estimation of the comb surface covered by bee bread cells using digital photography followed by image analysis

Indicator		**Assessing variable [unit]**	**Test method**
Quantity of bee bread		Total surface of combs covered by bee bread cells [cm^2]	Estimation of the comb surface covered by bee bread cells using digital photography followed by image analysis
Test characteristics		**Information on the method of analysis**	
Protocol	Test protocol	As detailed in Table C.7. Quantification of bee bread present in the hive combs by measuring the comb surface covered by bee bread, using digital photography followed by image analysis. There are three types of data output: 1) direct surface measurements (cm^2); 2) weight in kg (to calculate the weight a known surface area of bee bread is weighed and used as a reference to calculate the total quantity of bee bread, knowing the total surface area); 3) conversion of the surface area of bee bread into number of combs, using conversion values (e.g. table 2 in Delaplane, 2013)	
	Sampling (number, matrix, temporal and spatial aspects)	As detailed in Table C.7. After wintering, during the beekeeping season and before wintering. The period of prewintering is crucial because the quantity of bee bread is very important for successful resumption of the development and activity of the colony after the wintering period	
Evaluation		As detailed in Table C.8	

Table C.15: Estimation of the comb surface covered by bee bread cells using a transparent grid

Indicator		**Assessing variable [unit]**	**Test method**
Quantity of bee bread		Total surface of combs covered by bee bread [cm^2]	Estimation of the comb surface covered by bee bread cells using a transparent grid
Test characteristics		**Information on the method of analysis**	
Protocol	Test protocol	As detailed in Table C.8. Quantification of bee bread present in the hive combs by measuring the comb surface covered by bee bread using a transparent grid premarked in cm^2 and visually summing all the areas of bee bread.There are two types of data output: 1) direct surface measurements (cm^2); 2) weight in kg (to calculate the weight a known surface area of bee bread is weighed and used as a reference to calculate the total quantity of bee bread, knowing the total surface area	
	Sampling (number, matrix, temporal and spatial aspects)	As detailed in Table C.8. After wintering, during the beekeeping season and before wintering. The period of prewintering is crucial because the quantity of bee bread is very important for successful resumption of the development and activity of the colony after the wintering period	
Evaluation		As detailed in Table C.8	

Table C.16: Visual estimation of the number of combs covered by honey in the nest

Indicator		**Assessing variable [unit]**	**Test method**
Quantity of honey and nectar in the nest		Number of combs covered by honey and nectar in the nest [number of combs], [type of comb]	Visual estimation of the number of combs covered by honey and nectar in the nest
Test characteristics		**Information on the method of analysis**	
Protocol	Test protocol	As detailed in Table C.6. Estimate the percentage of comb surface occupied by honey. The observer is imaginatively sorting the resource into one contiguous mass and making a decision on the percentage surface area of the comb the contiguous resource occupies.There could be two types of data output: 1) number of combs of honey; 2) surface area (cm^2), using conversion values (e.g. table 2 in Delaplane, 2013)	
	Sampling (number, matrix, temporal and spatial aspects)	After wintering, during the beekeeping season and before wintering. The period of prewintering is crucial because the quantity of honey is very important for the successful resumption of the development and activity of the colony after the wintering period	
Evaluation		As detailed in Table C.6	

Table C.17: Estimation of the comb surface covered by honey using digital photography followed by image analysis

Indicator		**Assessing variable [unit]**	**Test method**
Quantity of honey and nectar in the nest		Total surface of combs covered by honey and nectar in the nest [cm^2]	Estimation of the comb surface covered by honey and nectar using digital photography followed by image analysis
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective	As detailed in Table C.7. Quantification of honey present in the hive combs by measuring the comb surface covered by honey, using digital photography followed by image analysis. There could be two types of data output: 1) direct surface measurements (cm^2); 2) weight in kg (to calculate the weight a known surface area of honey is weighed and used as a reference to calculate the total quantity of honey, knowing the total surface area)	
	Sampling (number, matrix, temporal and spatial aspects)	After wintering, during the beekeeping season and before wintering. The period of prewintering is crucial because the quantity of honey is very important for the production of heat during the winter	
Evaluation		As detailed in Table C.7	

Table C.18: Estimation of the comb surface covered by honey using a transparent grid

Indicator		**Assessing variable [unit]**	**Test method**
Quantity of honey and nectar in the nest		Total surface of combs covered by honey and nectar in the nest [cm^2]	Estimation of the comb surface covered by honey and nectar using a transparent grid
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective	As detailed in Table C.8. Quantification of honey and nectar present in the hive combs by measuring the comb surface covered by honey using a transparent grid premarked in cm^2 and visually summing the area of honey.There could be two types of data output: 1) direct surface measurements (cm^2); 2) weight in kg (to calculate the weight: weigh a known surface of honey and use it as a reference to calculate the total quantity of honey, knowing the total surface area).	
	Sampling (number, matrix, temporal and spatial aspects)	After wintering, during the beekeeping season and before wintering. The period of prewintering is crucial because the quantity of honey is very important for the production of heat during the winter	
Evaluation		As detailed in Table C.8	

Table C.19: Total concentration of pesticides in bee bread [μg/kg]

Indicator		**Assessing variable [unit]**	**Test method**
Pesticide contamination in bee bread		Total concentration of pesticides in bee bread (μg/kg (ppb))	Multiresidue analysis
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	Determining the total concentration of pesticides in bee bread using multiresidue analysis. The sampling can be performed by a beekeeper. The analysis step is carried out by specialised laboratories and comprises the extraction and identification of contaminants. Multiresidue techniques, such as the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe), can be used for the extraction of a wide range of contaminants in one analytical process (Bargańska et al., 2014). Frequently used analytical detection methods are gas chromatography coupled with time of flight mass spectrometry (GC-ToF), liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS), liquid chromatography coupled with mass spectrometry (LC–MS) and gas chromatography in combination with electronic capture detector (GC-ECD)	
	Sampling (number, matrix, temporal and spatial aspects	50 g of bee bread should be collected from randomly chosen cells. Pieces of combs with beebread must be collected from the brood chamber by cutting with a scalpel tool for example, put in a plastic box and shipped to a laboratory. An information sheet should be enclosed with the sample (not directly in contact with it), reporting the sampling date, type of sample, hive location and identification, beekeeper contact details and other information considered relevant	
Evaluation	Test sensitivity and LOD/LOQ	The LOD and LOQ vary according to the analysis technique used and the chemical compound detected, it is important to analyse the largest possible number of compounds in a given geographical context with the lowest LOQ and LOD, taking into account their toxicity for the bees (including sublethal). See Appendix E for more information. A (field) enquiry is needed to document the pesticides used and include these in the sampling plan	
	Test specificity	Test specificity will vary according to the analytical method used, but is in general considered to be high, particularly when the mass of molecules is determined (e.g. mass spectrometry)	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	Sampling can be done by every beekeeper and only requires some small equipment (scalpel, plastic box). The analysis can only be performed in a specialised laboratory, which is available in several EU Member States. The time, equipment and expertise required depend on the technique used. Samples must be sent to the laboratory in rigid, triple packaging in order to prevent crushing. Ensure that the cold chain remains unbroken until arrival at the laboratory	
	Standardisation	List of methods validated at the EURL according to French (AFNOR V03-110 Standard) or international standards (SANCO/12571/2013): • Determination of neonicotinoid residues in bee bread by LC–MS/MS. • Determination of neonicotinoid residues in pollen by LC–MS/MS. • Determination of organochlorine, organophosphorus, pyrethroid pesticide residues in honeybees by GC (multiresidue method). • In progress, following the same standards: search for pesticides (organochlorines, organophosphorus, pyrethroids and dicarboximide) in bee bread by GC (multiresidue method)	
	Possible limitations to use in field survey	Sampling can be performed by a beekeeper but the analysis must be performed in a specialised laboratory. For the most toxic molecules, analysis of individual molecules is required to achieve a sufficiently low LOD and LOQ	

GC: gas chromatography; GC–ECD: gas chromatography in combination with electronic capture detector; GC–ToF: gas chromatography coupled with time of flight mass spectrometry; LC–MS: liquid chromatography coupled with mass spectrometry; LC–MS/MS: liquid chromatography coupled with tandem mass spectrometry; LOD: limit of detection; LOQ: Limit of quantification.

Table C.20: Total concentration of pesticides in wax [μg/kg]

Indicator		**Assessing variable [unit]**	**Test method**
Pesticide contamination in wax		Total concentration of pesticides in wax [μg/kg (ppb)]	Multiresidue analysis
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	As detailed in Table C.19	
	Sampling (matrix, number, temporal and spatial aspects (sampling)	Sample ~ 50 g of wax. Label and send samples in a clean cardboard or paper recipient. Avoid using plastic as well as crushing the samples. Combs must be collected from the brood chamber. Sampling on different combs at the centre and at the border of the combs are required to avoid the heterogeneity of this complex matrix. This complexity of the matrix could result in a high heterogeneity in the results	
Evaluation	Test sensitivity and LOD/LOQ	Similar to Table C.19. See Appendix E for more information	
	Test specificity	As detailed in Table C.19	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	As detailed in Table C.19	
	Standardisation	See Appendix E	
	Possible limitations to use in field survey	As detailed in Table C.19	

LOD: limit of detection; LOQ: Limit of quantification.

C.1.4. Behaviour and physiology of the bees

Table C.21: Visual estimation of the number of returning foragers

Indicator		**Assessing variable [unit]**	**Test method**
Foraging activity		Foraging rate – average number of returning foragers per time unit, assessed by visual observation [number/min in the morning; number/min in the early afternoon]	Visual estimation of the number of returning foragers
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	To determine the average number of foraging events (i.e. returning foragers) per unit time. Returning foragers are counted over 10 min. The average number of forager bees returning per minute is then calculated. If foraging is chronically low, this might suggest a problem for the colony. However, it is also possible that there may be short-term drops in foraging that are normal (i.e. short-term shortage of pollen in the landscape, a change in weather, high winds)	
	Sampling (matrix, number, temporal and spatial aspects)	Adult bees returning to the hive. Two 10-min samples, one in the late morning and one in early afternoon, with 4 h difference between the two observations, which allows for some standardisation. Some hives may naturally be more active in the morning or in the afternoon, depending on the flowering phenology of the landscape (see, Abou-Shaara, 2014). Variability is normal and often follows the pattern of low foraging rates in the early spring, high rates in summer and medium rates in the autumn. Data should be collected in the spring (specifically at the end of winter), summer and autumn (during the autumn bloom while the hive is still foraging). Care should be taken that the observer does not disturb the returning bees (i.e. stands to the side of hive entrance) (Delaplane et al., 2013a,b)	
Evaluation	Test sensitivity and LOD/LOQ	Test sensitivity is assumed to be high because the numbers to count are relatively low (e.g. 17.66 and 36.02 foragers/min have been reported, see Abou-Shaara, 2014). Variation between observers is considered low because it is easy to recognise returning foragers	
	Test specificity	Test specificity is assumed to be high. Returning foragers are a robust and easy to recognise variable to estimate	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	An estimation can be made by counting the number of returning foragers per unit time and only requires short training to recognise returning foragers. Clickers may facilitate the counting, but are not necessary. Formal counting is not routinely performed by beekeepers across the EU except in a research setting, although beekeepers informally notice if a hive is actively foraging or not	
	Standardisation/ validation	Care should be taken that these data are obtained during good weather conditions: minimum of 10°C, low wind and an absence of rainfall. If two observers are present, then the counting may be more accurate (sensitive), and a per minute and overall average could be obtained, with a good understanding of the variability (Delaplane et al., 2013a,b; Riddell Pearce et al., 2013). Data interpretation however, should include season, weather description and size of hive	
	Possible limitations to use in field survey	There is no practical limitation to implement this method in field surveys	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.22: Visual identification of atypical worker behaviour by checking through several combs

Indicator		**Assessing variable [unit]**	**Test method**
Atypical workers behaviour		Level of atypical behaviour of workers inside the hive [percentage]	Visual identification of atypical worker behaviour inside the hive
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	Visual inspection of the worker bees on several combs is carried out to assess the presence/absence of the worker bees in the hive showing atypical behaviour (see Appendix F for more details on atypical behaviours)	
	Sampling (matrix, number, temporal and spatial aspects)	Preferably every time the hive is opened during routine inspection, at least a minimum of three inspections during a year (spring, summer and autumn). A majority (> 60%, including brood comb) of frames containing workers inside the hive should be inspected	
Evaluation	Test sensitivity and LOD/LOQ	Test sensitivity is assumed to be high because worker behaviours are robust and stereotyped. Atypical behaviours in the hive are easily recognised by beekeepers, especially the more experienced ones. The probability of missing abnormal behaviours in the hive is presumed to be low. Anything below 5% would be hard to detect, and above 15%, it would be harder to determine exact levels	
	Test specificity	Test specificity is assumed to be high because worker behaviours are easy to recognise, stereotyped and robust, and even new beekeepers are soon familiar with the suite of normal behaviours	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	Is currently routinely done by most beekeepers throughout the EU. Worker bee behaviour can be assessed whenever a beekeeper is opening hives for routine inspection. Time of observation will be ~ 1 min per frame. No equipment is required except the standard beekeeping equipment used to open and to inspect the inside of a hive. No particular training is required	
	Standardisation/ validation	The method not standardised (refer to a beekeeping manual, e.g. Winston, 1991) but it is considered that data on observed abnormal behaviour of worker bees could be merged	
	Possible limitations to use in field survey	There is no practical limitation on implementing this method in a field survey	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.23: Visual estimation of atypical worker behaviour in the vicinity of the hive

Indicator		**Assessing variable [unit]**	**Test method**
Atypical worker behaviour		Level of atypical behaviour of workers in the vicinity of the hive [percentage]	Visual estimation of atypical worker behaviour in the vicinity of the hive
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	Visual inspection of the worker bees in the vicinity (radius 2 m) of the hive to estimate the percentage of worker bees showing atypical behaviours. If one observes many (e.g. > 15%) or several (e.g. 5–15%) workers engaging in atypical behaviours (including unusual inactivity), this is an indication that the colony is not healthy. If one observes few (e.g. < 5%) bees engaging in atypical behaviour, this may represent a background level and could be normal. However, in this case, the hive should be monitored. If most (e.g. 95% or more) of the workers are engaged in typical behaviours, this is an indication that the colony is healthy	
	Sampling (matrix, number, temporal and spatial aspects)	The behaviour of the workers within a 2 m radius around the hive should be inspected. Every time the beekeeper is in the apiary for routine inspections, a minimum of three inspections during a year (spring, during the beekeeping season and autumn)	
Evaluation	Test sensitivity and LOD/LOQ	Test sensitivity is assumed to be high because worker behaviours are robust and stereotyped. Atypical behaviours around the hive are easily recognised by beekeepers, especially the more experienced ones. The probability of missing abnormal behaviours around the hive is presumed to be low. Probably anything below 5% would be hard to detect, and above 15%, it would be harder to determine levels. However, care should be taken that the observation is careful and includes on the ground, in the grass, below the entrance, on the entrance board and on the stones within a 2 m radius of the hive	
	Test specificity	Test specificity is assumed to be high because worker behaviours are easy to recognise, stereotyped and robust, and even new beekeepers are usually soon familiar with the suite of normal behaviours	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	Is currently routinely done by most beekeepers throughout the EU, although informally. Can be done whenever a beekeeper is walking near the hive during routine inspection and does not require that the hive be disturbed. The time required is a few minutes per hive, and no special training or dissemination of method is required	
	Standardisation/ validation	Method not standardised (refer to a beekeeping manual, e.g. Winston, 1991). Implementation of a standard operating procedure describing which places to check (e.g. entrance board, ground within a 2 m radius around the hive, flying bees, etc.) would improve the quality of the collected data	
	Possible limitations to use in field survey	There is no practical limitation to implement this method in a field survey	

LOD: limit of detection; LOQ: Limit of quantification.

C.1.5. Disease, infection and infestation

Table C.24: Visual inspection of the hive for clinical signs detection

Indicator		**Assessing variable [unit]**	**Test method**
Clinical signs		Presence of clinical signs	Visual inspection of the hive
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	Visual observation of the colony to assess the presence of clinical signs in brood and adult bees. For assessment of brood, shaking all the bees off the brood comb will allow an unimpaired view of sealed and unsealed brood cells. A detailed list of the clinical signs of the diseases most frequently observed in European honeybee colonies can be found in Appendix D	
	Sampling (matrix, temporal and spatial aspects)	The inspection can be done whenever the beekeeper inspects the colony, but the inspection should take place three times a year at least (after winter, during the beekeeping season and before winter). The clinical signs vary with the disease and some may be more easily observed at certain times of the year (e.g. Varroosis clinical signs are typically observed at the end of beekeeping season)	
Evaluation	Test sensitivity and LOD/LOQ	Test sensitivity will depend on the level of infestation and on the observer's ability to recognise clinical signs in adult bees or brood	
	Test specificity	The majority of the clinical signs are not specific to a single disease and therefore a differential diagnosis is needed, using laboratory techniques, to identify the agent(s) causing disease	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	For an optimal examination of the hive, 15–20 min per hive may be necessary. It is assumed that the results will have less variability if the beekeepers undergo short training to learn how to recognise clinical signs. Only standard equipment for opening hives (veil, smoker, hive tool) is needed	
	Standardisation	It is not a standardised method although it is used in an informal way by most beekeepers. For reporting of this variable, it is recommended to use the clinical signs checklist available in Appendix D, and use the terminology mentioned in Figure 8 (Section 3.2.5 in main text)	
	Possible limitations to use in field survey	No practical limitations were identified	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.25: Capturing and counting *Varroa* mites using a sticky trap after natural fall

Indicator		**Assessing variable [unit]**	**Test method**
Varroa		*Varroa* infestation level in hive by natural fall using sticky traps [number of mites in the sticky traps]	Capturing and counting *Varroa* mites using a sticky trap after natural fall
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	Placing a sticky trap in the bottom of the hive and counting the number of *Varroa* mites that naturally fall onto the sheet. This method provides a reliable estimate of infestation as long as the colony is producing brood and not collapsing (Branco et al., 2006; Flores et al., 2015)	
	Sampling (matrix, temporal and spatial aspects)	Debris collected in the sticky trap. One sticky board should be placed in the hive bottom over 4 days (if the sticky board is left there longer than the debris can impair mite counting) (Flores et al., 2015), fully covering the hive bottom, twice a year (first visit after winter and at the end of beekeeping season). In particular, assessing the level of infestation at the end of the beekeeping season is important to ensure that winter bees will be born in good condition	
Evaluation	Test sensitivity and LOD/LOQ	Test specificity is assumed to be moderate. It will vary according to the beekeeper's ability to identify the mites among the sticky trap debris	
	Test specificity	The *Varroa* mite may be confused with the bee louse *Braula coeca* (World Organisation for Animal Health (OIE), 2008a,b,c). However, it is assumed that most European beekeepers will be able to discriminate *Varroa* from *Braula coeca*. Samples can be sent to a laboratory for confirmation, in particular in areas where the mite is not yet reported	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	The equipment required is a screened floor board and a sticky trap. Sticky traps are commercially available or can be self-made and should entirely cover the bottom of the hive. Identification and counting of *V. destructor* can be done in the field by beekeepers who received training to recognise the morphological characteristics of the parasite. The time required to count the mites will depend on the hive infestation level. In highly infested colonies counting all mites may be a very laborious task and may provide very inconsistent results. If > 1,000 mites are present it is sufficient to count a quarter of the cells to obtain a reliable figure (Dietemann et al., 2013). It is also possible to take the trap home to count the mites afterwards, possibly obtaining a more consistent result	
	Standardisation	The repeatability and reproducibility of capturing and counting *Varroa* mites using sticky traps are low because many different types of sticky traps are available. In highly infested colonies, variation in the results may be decreased by previously drawing a grid in the sticky sheet to help mite counting. The method is described in Dietemann et al. (2013) but is not standardised	
	Possible limitations to use in field survey	No practical limitations were identified	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.26: Counting the number of mites dislodged with alcohol

Indicator		**Assessing variable [unit]**	**Test method**
Varroa		*Varroa* infestation level on adult a bees after bee submersion in alcohol [number of mites], [number of bees]	Counting the number of mites dislodged with alcohol
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	Identification and counting the number of *Varroa* mites dislodged with alcohol from at least 200–250 adult bees (Fries, 1991). For further calculation of the level of infestation, both the number of sampled adult bees and the number of counted mites should be reported. When sampling adult bees, it is best to locate the queen to assure that she does not accidentally end up among the sampled bees	
	Sampling (matrix, temporal and spatial aspects)	At least 200–250 adult bees collected from at least three brood combs. The infestation level should be assessed at the end of the beekeeping season	
Evaluation	Test sensitivity and LOD/LOQ	The sensitivity of this method is low because only a small proportion of the bees in the hive are sampled (200–250 out of the total number of bees in the colony). The method is more precise in brood-rearing colonies	
	Test specificity	The *Varroa* mite may be confused with the bee louse *Braula coeca* (World Organisation for Animal Health, 2008b). It is assumed that most European beekeepers will be able to discriminate *Varroa* from *Braula coeca*. Samples can be sent to a laboratory for confirmation	
	Required time, equipment, expertise and availability/ dissemination of method (sampling/analysis)	A jar and a 75% alcohol in water solution are needed. The container should be stirred for 10 min (World Organisation for Animal Health, 2008b). It is frequently applied by beekeepers. Identification and counting of *V. destructor* can be done in the field by beekeepers who received training to recognise the morphological characteristics of the parasite	
	Standardisation	The method is not standardised and it is used in an informal way by beekeepers. The method is described by Dietemann et al. (2013) but is not standardised	
	Possible limitations to use in field survey	No practical limitations were identified	

LOD: limit of detection; LOQ: Limit of quantification.

Table C.27: Identification of the species *Paenibacillus larvae* through (conventional and real-time) polymerase chain reaction

Indicator		**Assessing variable [unit]**	**Test method**
Paenibacillus larvae (AFB)		Identification of the species *P. larvae*	Identification of the species *P. larvae* through (conventional and real-time) PCR on larvae showing clinical signs (in the presence of clinical signs) or in adult bees/honey/debris (in the absence of clinical signs)
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective and description	To identify the species *P. larvae* from diseased larvae (conventional PCR) and the quantification of the spores (real-time PCR). Use of the real-time PCR is preferred. If no clinical signs are observed, the sampling matrix should be adult bees, honey or debris	
	Sampling (matrix, temporal and spatial aspects)	If clinical signs are observed, a 10 × 10 cm piece of brood containing at least 15 larvae and/or pupae with signs of disease or infected larval/pupal is collected directly from the cells using a sterile swab. The sampled brood comb should be wrapped in a paper bag, paper towel or newspaper and placed in a wooden or heavy cardboard box for transport; any form of plastic wrapping should be avoided to prevent fungal growth. Swabs with larval remains can be put into appropriate test tubes with a cap (World Organisation for Animal Health, 2008). If it is not possible to send the comb immediately, the sample should be kept fresh by chilling in a refrigerator. After cutting the comb, thoroughly clean the knife to avoid possible spread of disease to other colonies. This method has to be applied on larvae showing clinical signs. It is not adapted for systematic analyses. Therefore, no temporal aspect can be specified. If no clinical signs are observed the following should be sampled: honey – at least 20 g; adult bees – at least 100 bees (it is better to have between 300 and 500 bees, but the sampling size can be adapted according to the strength of the colony); debris, which can be collected by placing a grid floor and a sticky trap (debris to be collected after several days)	
Evaluation	Test sensitivity and LOD/LOQ	High sensitivity and high specificity	
	Test specificity	PCR is more specific than other methods (e.g. bacterial culture or microscopy) for *P. larvae* identification. The primers designed by Govan et al. (1999) and Dobbelaere et al. (2001), which are recommended in the World Organisation for Animal Health (OIE) (2008c), have been tested with 14 different *Paenibacillus* or *Bacillus* species	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	Beekeepers can take samples and send these for analysis in a laboratory equipped for molecular biology analyses. There are several laboratories within the EU that already apply conventional PCR and a smaller number that apply real-time PCR	
	Standardisation	To date, there is no standardised method. Methods are described in the World Organisation for Animal Health (OIE) (2008c) and harmonisation procedures are ongoing	
	Possible limitations to use in field survey	No practical limitations were identified	

LOD: limit of detection; LOQ: Limit of quantification; PCR: polymerase chain reaction.

C.2. External drivers

C.2.1. Resource providing unit

Table C.28: Example of hierarchical LAND COVER/LAND USE classification

LEVEL 1 (CORINE land cover)	LEVEL 2 (CORINE land cover)	LEVEL 3 (CORINE land cover)	LEVEL 4
Artificial surfaces	Urban fabric	Continuous urban fabric	Brown fields
			Actively used
Artificial surfaces	Urban fabric	Discontinuous urban fabric	Brown fields
			Actively used
Artificial surfaces	Industrial, commercial and transport units	Industrial or commercial units	
Artificial surfaces	Industrial, commercial and transport units	Road and rail networks and associated land	Brown fields
			Actively used
Artificial surfaces	Industrial, commercial and transport units	Port areas	
Artificial surfaces	Industrial, commercial and transport units	Airports	
Artificial surfaces	Mine, dump and construction sites	Mineral extraction sites	Brown fields
			Actively used
Artificial surfaces	Mine, dump and construction sites	Dump sites	Brown fields
			Actively used
Artificial surfaces	Mine, dump and construction sites	Construction sites	Brown fields
			Actively used
Artificial surfaces	Artificial, non-agricultural vegetated areas	Green urban areas	Urban agriculture
			Flower strips
Artificial surfaces	Artificial, non-agricultural vegetated areas	Sport and leisure facilities	Recreational parks
			Sports parks
			Gardens
Agricultural areas	Arable land	Non-irrigated arable land	Resourceful crops (e.g. oil seed rape, sunflower, beans)
			Non-resourceful crops (e.g. wheat, sugar beet, barley)
			Flower strips and hedgerows

LEVEL 1 (CORINE land cover)	LEVEL 2 (CORINE land cover)	LEVEL 3 (CORINE land cover)	LEVEL 4
Agricultural areas	Arable land	Permanently irrigated land	Resourceful crops (e.g. maize, sunflower, beans)
			Non-resourceful crops (e.g. sugar beet)
			Flower strips and hedgerows
Agricultural areas	Arable land	Rice fields	Rice fields
			Flower strips and hedgerows
Agricultural areas	Permanent crops	Vineyards	Vineyards
			Flower strips and hedgerows
Agricultural areas	Permanent crops	Fruit trees and berry plantations	Traditional orchards (vegetation under the trees, high trees)
			Conventional orchards (less vegetation under the trees, smaller trees, higher density of trees)
			Enclosed farming (glass houses)
			Open berry plantation
			Semienclosed farming
			Flower strips and hedgerows
Agricultural areas	Permanent crops	Olive groves	Traditional orchards (vegetation under the trees, high trees)
			Conventional orchards (less vegetation under the trees, smaller trees, higher density of trees)
			Flower strips and hedgerows
Agricultural areas	Pastures	Pastures	Improved grassland (low number of species)
			Non-improved grassland (high number of species)
			Monoculture (e.g. alfalfa)
			Flower strips and hedgerows
Agricultural areas	Heterogeneous agricultural areas	Annual crops associated with permanent crops	Permanent and annual flowering crops
			Permanent and annual non-flowering crops
			Flower strips and hedgerows
Agricultural areas	Heterogeneous agricultural areas	Complex cultivation patterns	Permanent and annual flowering crops
			Permanent and annual non-flowering crops
			Flower strips and hedgerows

Honeybee colony health (HEALTHY-B)

LEVEL 1 (CORINE land cover)	LEVEL 2 (CORINE land cover)	LEVEL 3 (CORINE land cover)	LEVEL 4
Agricultural areas	Heterogeneous agricultural areas	Land principally occupied by agriculture, with significant areas of natural vegetation	Permanent and annual flowering crops
			Permanent and annual non-flowering crops
			Flower strips and hedgerows
			Flowering natural vegetation
			Non-flowering natural vegetation
Agricultural areas	Heterogeneous agricultural areas	Agroforestry areas	Tree species (broadleaves, coniferous, mixed forest)
			Flowering crop
			Non-flowering crop
			Flowering wild vegetation
			Non-flowering wild vegetation
			Flower strips and hedgerows
Forest and seminatural areas	Forests	Broadleaved forest	Flowering wild vegetation
			Non-flowering wild vegetation
Forest and seminatural areas	Forests	Coniferous forest	Flowering wild vegetation
			Non-flowering wild vegetation
Forest and seminatural areas	Forests	Mixed forest	Flowering wild vegetation
			Non-flowering wild vegetation
Forest and seminatural areas	Scrub and/or herbaceous vegetation associations	Natural grasslands	Nutrient rich (higher biodiversity)
			Nutrient poor (lower biodiversity)
Forest and seminatural areas	Scrub and/or herbaceous vegetation associations	Moors and heathland	
Forest and seminatural areas	Scrub and/or herbaceous vegetation associations	Sclerophyllous vegetation	
Forest and seminatural areas	Scrub and/or herbaceous vegetation associations	Transitional woodland-shrub	
Forest and seminatural areas	Open spaces with little or no vegetation	Beaches, dunes, sands	
Forest and seminatural areas	Open spaces with little or no vegetation	Bare rocks	
Forest and seminatural areas	Open spaces with little or no vegetation	Sparsely vegetated areas	
Forest and seminatural areas	Open spaces with little or no vegetation	Burnt areas	
Forest and seminatural areas	Open spaces with little or no vegetation	Glaciers and perpetual snow	

LEVEL 1 (CORINE land cover)	LEVEL 2 (CORINE land cover)	LEVEL 3 (CORINE land cover)	LEVEL 4
Wetlands	Inland wetlands	Inland marshes	Non-flowering wild vegetation
			Flowering wild vegetation
Wetlands	Inland wetlands	Peat bogs	Non-flowering wild vegetation
			Flowering wild vegetation
Wetlands	Maritime wetlands	Salt marshes	Non-flowering wild vegetation
			Flowering wild vegetation
Wetlands	Maritime wetlands	Salines	
Wetlands	Maritime wetlands	Intertidal flats	
Water bodies	Inland waters	Water courses	Canals (irrigation, drainage)
			streams, rivers
Water bodies	Inland waters	Water bodies	Lakes, ponds
Water bodies	Marine waters	Coastal lagoons	
Water bodies	Marine waters	Estuaries	
Water bodies	Marine waters	Sea and ocean	

Measuring floral resources in the landscape requires four steps:

(1) Measure the number of open flowers in each habitat at a given point.
(2) Sample nectar (including water and energy).
(3) Sample pollen.
(4) Extrapolate upwards.

Table C.29: Measuring pollen and nectar resources in a landscape

Steps	Test method	Notes
1) Measuring the number of open flowers	A representative sample of the floral resources within a habitat can be taken by randomly placing at least three quadrats (1 m × 1 m or larger) within a habitat, or along a transect walk path of random origin with quadrats placed periodically.Within each quadrat the number of floral resource units per species should be counted.These floral resource units are defined (taken from Carvell et al., 2007) as either a single flower or, where plants have multiflowered stems: • an umbel (e.g. *Daucus carota*); • a head (e.g. *Trifolium pratense*); • a spike (e.g. *Rhinanthus minor*); • a capitulum (e.g. *Centaurea nigra*). The proportion of open flowers can be counted by dissecting at least three typical floral resource units to estimate the proportion of opened flowers at that time. This can in turn be averaged per species across all quadrats.	The number of open flowers determines the global availability of floral resources; the number of flowers alone will overestimate available resources at a given time and may ignore floral losses.
2) Measuring nectar resources (i.e. nectar productivity)	Ideally flowers should be covered to prevent nectar loss from pollinators, or sampling can take place before pollinator activity (e.g. Potts et al., 2006). Open flowers can also be used because there is a significant positive correlation between open and bagged plant nectar resources. Nectar extracted from closed flowers was, in a Mediterranean system, found to contain ~ 3.3 times as much energy as open flowers (Potts et al., 2003b). Full extraction protocol (taken from Human et al., 2013). Note that step 7 is important only if the nectar is to be analysed for chemical residues afterwards. Water content can also be measured as part of step 6. Sucrose can be converted into energy using standard calorific conversion measures. 1) Cover flowers to be examined with gauze (2 mm mesh size) to exclude visitation of any pollinators. 2) Remove flower petals gently to reveal nectar at the base of the flowers. 3) Withdraw/collect the nectar from the flower in disposable microcapillary tubes (length 75 mm, capacity 75 μL) by capillary attraction. 4) Determine volumes of nectar from column length in the microcapillary tubes (75 mm is equivalent to 75 μL). 5) Release the nectar onto the prismatic surface of a pocket refractometer. 6) Measure the nectar concentration as per cent (w/w) sucrose equivalents.	Nectar is the principle source of honeybee energy and is the primary material in honey production. It also acts as a major source of water. Nectar can be extracted from live honeybees (see Human et al., 2013) but this is not appropriate for assessing nectar resources in a particular landscape

Steps	Test method	Notes
	7) Depending on the purpose of nectar collection, samples should either be used immediately in the field or transported to the laboratory on either dry ice or on filter paper (Whatman no. 1) (Dafni et al., 2005) after which it should be stored in 15 mL centrifuge tubes at –20°C until ready for composition or residue analysis. Alternatively, assessing the nectar collected by honeybees can be done in a non-fatal way using the following protocol from Human et al. (2013); however, this is likely to miss some of the nectar collected because not all will be regurgitated and some will be consumed during flight. 1) Capture honeybees visiting flowers on the plant of interest or at the entrance of hives on their way back from nectar gathering. 2) Compress the thorax of individual bees gently dorsoventrally to obtain nectar to induce regurgitation of the content of the honey stomach (Roubik and Buchmann, 1984). This should be done within 10 min of capture, to prevent the honeybee using her stomach load as fuel. 3) Collect the liquid nectar from the mouthparts in microcapillary tubes through capillary action. 4) Measure nectar volume. Volumes (μL) are determined from the column length in microcapillary tubes (length 75 mm/75 μL). 5) Measure nectar concentration with a pocket refractometer (e.g. Bellingham and Stanley Ltd, Tunbridge Wells, UK) by placing a drop of nectar onto the prismatic surface of the refractometer (through capillary action). Concentration is measured as per cent (w/w) sucrose equivalents.	
3) Measuring pollen resources (i.e. pollen productivity)	It is generally advisable to enclose sample plants to be assessed for pollen content using gauze to exclude insect visitation because insects can remove large quantities of pollen at a time.Pollen can be collected in a number of ways. On large flowers (e.g. maize) – passively, using wax papers bags tied to floral units prior to anthesis (this is only suitable for plants that produce a lot of light pollen), shaking pollen from plants onto trays or brushing pollen off using a paintbrush, etc. (Human et al., 2013). On smaller flowers – invasively in a laboratory using the protocols given below. Protocol described by Human et al. (2013): 1) Collect flower clusters in the early morning when plants are 40–50% flowering. 2) Place the clusters into containers. 3) Allow the clusters to dry at a processing location. 4) Brush flowers over food strainers to separate pollen from anthers.	Standard calorific and protein analyses can be used to estimate the nutritional content of the pollen, although some past studies (e.g. Petanidou and Vokou, 1990) can provide estimates of the energy content per g of pollen. Assessment of the pollen collected by honeybees can be easily undertaken using commercial pollen traps placed at the entrance to hives (Human et al., 2013) and following steps 5 and 6 of the Potts et al. (2003a) protocol below.

Steps	Test method	Notes
	5) Clean samples of pollen by sifting through multiple sieves of different pore sizes (pore sizes: 0.119 and 0.0043 cm). 6) Store collected pollen at –20°C until ready for further testing. Protocol given by Potts et al. (2003a): 1) Removing all anthers that open (either freshly dehisced or previously open) and storing them in 70% alcohol (Human et al. recommend doing this early in the morning when flowers are 40–50% open). 2) Sonicate the anthers for 10 min. 3) Filter the sonicated mixture through a 0.1 mm mesh to remove plant debris. 4) Centrifuge the remaining mixture. 5) Using light microscopy, estimate the number of pollen grains and mean grain size. 6) The total mas of pollen per unit area can then be calculated using regression models such as Roulston and Cane, (2000).	
4) Extrapolation	Nectar and pollen production will be influenced by soil, rainfall, etc. because these affect plant fecundity. Nectar and pollen will also vary within the year depending on the time during plant flowering. Plant communities can be highly variable across a landscape even within similar habitats (this can be solved by increased sampling or by making a note of the species diversity that is not sampled in quadrats).	Once estimated for a representative sample of each habitat, the pollen and nectar resources of open flowers can be upwardly extrapolated to the designated landscape area.

C.2.2. Environmental drivers

Table C.30: Environmental drivers

Factor	Source	Temporal resolution	Spatial resolution	Comments
Weather factors				
Temperature and precipitation	E-OBS gridded data set: http://www.ecad.eu/download/ensembles/download.php#months AGRI4MARS: http://agri4cast.jrc.ec.europa.eu/DataPortal/Index.aspx?o=d Reference: E-OBS temperature and precipitation: 'We acknowledge the E-OBS data set from the EU-FP6 project ENSEMBLES (http://ensembles-eu.metoffice.com) and the data providers in the ECA&D project (http://www.ecad.eu)' Haylock MR, Hofstra N, Klein Tank AMG, Klok EJ, Jones PD and New M, 2008. A European daily high-resolution gridded data set of surface temperature and precipitation. Journal of Geophysical Research: Atmospheres, 113, D20119. doi: 10.1029/2008JD10201	Daily	0.25° and 0.50° on a regular grid. Rotated pole grid also available, at 0.22° and 0.44°.	Gridded observational data set for precipitation, temperature (and sea-level pressure) in Europe.
Solar radiation	EUMETSAT Satellite Application Facility on Climate Monitoring (CM SAF) https://climatedataguide.ucar.edu/climate-data/surface-solar-radiation-europe-africa-and-atlantic-based-mviri-visible-channnels	Hourly	0.03°	
Relative humidity	Vmerge project: emerging viral vector-borne disease. Jones P and Wint W, 2015. Data set produced by Waen Associates for Environmental Research Group Oxford, Limited, funded by the International Research Consortium on Dengue Risk Assessment, Management and Surveillance (IDAMS), European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 281803. http://www.edenextdata.com/?q=data NOAA database: http://www.esrl.noaa.gov/psd/data/data_faq.html		5 or 10 km	Spatial resolution depending on geographic region.
Climate factors				
Snow cover	SnowClim project (snow climate monitoring): www.dwd.de/snowclim http://www.emetsoc.org/fileadmin/ems/dokumente/annual_meetings/2010/MC3_Bissolli.pdf		0.1° (~ 12.5 km)	Interpolated data from station snow data
	MODIS/Terra Snow Cover Daily L3 Global 500 m Grid, Version 5:https://nsidc.org/data/mod10a1 Satellite data	Daily observation from 2000 to present	500 m	Unprocessed data
	EURO-CORDEX simulated data, for climate projections for different Representative Concentration Pathways: http://www.euro-cordex.net/EURO-CORDEX-Data.2613.0.html	Daily	0.1° (~ 12.5 km)	Unprocessed data

Factor	Source	Temporal resolution	Spatial resolution	Comments
Climate type (average precipitation, average temperature, thermal sums)	Peel MC, Finlayson BL and McMahon TS, 2007. Updated world map of the Koppen–Geiger climate classification. Hydrology and Earth System Sciences, 11, 1633–1644. doi: 10.5194/hess-11-1633-2007 http://www.hydrol-earth-syst-sci.net/11/1633/2007/hess-11-1633-2007.pdf			ESRI grid data available at: http://www.hydrol-earth-syst-sci.net/11/1633/2007/hess-11-1633-2007-supplement.zip Thermal sums to be calculated

C.2.3. Beekeeping management practice

Table C.31: Beekeeping management practice

Factor	Variable	Used in previous studies	Notes	Target (beekeeper, inspector, or it can be derived from other variable/s within the beekeeping record book[(c)])
Experience	Training information • Number of beekeeping courses attended. • Bee meetings attended. • Qualification obtained. • Membership of beekeeping association (low score on its own). • Years of practice. Technical abilities • Age of the beekeeper. • Knowledge of bee pests/parasites/diseases biology identification and control methodologies. • Knowledge of bee biology. • Knowledge of environmental stressors. • Colony survival rate /year control methodologies.	Yes EPILOBEE 2012–2014 (2015); Deloitte (2013)	In EPILOBEE beekeepers are characterised essentially based on the number of colonies and apiaries being managed and whether the activity of the beekeeper is generating an income. In Deloitte (2013) the focus is given on the years of experience of the beekeepers.	Beekeeper /Inspector
Chemical control method Note: A preselection of active ingredients was used in the majority of the questionnaires.	Per each active ingredient provide the dates of treatments and respective target (specifying the dose, application method, duration of treatment).	Yes :Brodschneider and Crailsheim (2010); EPILOBEE 2012–2014; NBU questionnaire 2014; Van der Zee et al. (2013); COLOSS questionnaire, 2015; BeeNet	Exact date of the treatment was not present (COLOSS[(a)])BeeNet[(b)]	Beekeeper

Factor	Variable	Used in previous studies	Notes	Target (beekeeper, inspector, or it can be derived from other variable/s within the beekeeping record book[(c)])
Physical control	Number of physical control events/year	No	NA	Derived from other variable/s within the beekeeping record book[(c)]
	• *Varroa* control methods with no chemicals used: worker brood removal [Y/N]	Yes:COLOSS; BeeNet	COLOSS groups queen confinement, trapping comb, complete brood removal, etc. as 'other biotechnical methods' BeeNet[(b)]	Beekeeper
	• *Varroa* control methods with no chemicals used: drone brood removal [Y/N]	Yes: COLOSS; BeeNet	COLOSS groups queen confinement, trapping comb, complete brood removal, etc. as 'other biotechnical methods' BeeNet[(b)]	Beekeeper
	• *Varroa* control methods with no chemicals used: queen confinement [Y/N]	Yes: COLOSS; BeeNet	COLOSS[(a)] groups queen confinement, trapping comb, complete brood removal, etc. as 'other biotechnical methods' BeeNet[(b)]	Beekeeper
	• *Varroa* control methods with no chemicals used: heat treatment of brood/bees (i.e. hyperthermia) [Y/N]	Yes: COLOSS; BeeNet	COLOSS[(a)] BeeNet[(b)]	Beekeeper
	Trapping effort: total number of days of trapping $\sum_i(Ti \times t)$ (trapping effort)	No	NA	Derived from other variable/s within the beekeeping record book[(c)]
	• SHB at colony level *Ti*	No	Invasive species officially reported in part of an EU country (one, Italy)	Beekeeper
	• *Vespa velutina*: at apiary level *Tj*	No	Invasive species officially reported in part of EU countries (five)	Beekeeper

Factor	Variable	Used in previous studies	Notes	Target (beekeeper, inspector, or it can be derived from other variable/s within the beekeeping record book[(c)])
	• Other *Vespa* (i.e. *V. orientalis*): at apiary level *Tk*	No	NA	Beekeeper
	Other methods for *Vespa* spp. (will be addressed in a simplified manner through the use of a questionnaire only)	No	NA	Beekeeper
	Non-living material sterilisation of contaminated material by:			
	• Gamma-rays [Y/N]	No	NA	Beekeeper
	• Heat treatment by flames [Y/N]	No	NA	Beekeeper
	• No sterilisation, but disposal [Y/N]	No	NA	Beekeeper
	• Neither sterilisation nor disposal [Y/N]	No	NA	Beekeeper
	• Steam?	No	NA	Beekeeper
	Storage of beekeeping materials including supers and frames			
	• Closed and uncontaminated facilities [Y/N]	No	NA	Beekeeper
	• Refrigerated facilities [Y/N]	No	NA	Beekeeper
	• Controlled atmosphere facilities CO_2 treatment [Y/N]	No	NA	Beekeeper

Factor	Variable	Used in previous studies	Notes	Target (beekeeper, inspector, or it can be derived from other variable/s within the beekeeping record book[c])
Replacement combs with brood	Date and number of brood combs introduced per colony/year	Yes:BeeNet	BeeNet[b]	Beekeeper
	Date and number of brood combs removed per colony/year	Yes:COLOSS questionnaire, 2015; NBU questionnaire, 2014 BeeNet	BeeNet[b] Proportion of brood combs replaced is included in COLOSS questionnaire, 2015; NBU questionnaire, 2014	Beekeeper
	Number of brood combs introduction events per colony/ year	No	NA	Derived from other variable/s within the beekeeping record book[c]
	Number of brood combs removal events per colony/year	No	NA	Derived from other variable/s within the beekeeping record book[c]
Replacement combs with food sources	Date and number of food combs introduced per colony/year	Yes:BeeNet	BeeNet[b]	Beekeeper
	Date and number of food combs removed per colony/year	Yes:BeeNet	BeeNet[b]	Beekeeper
	Number of food comb introduction events per colony/year	No	NA	Derived from other variable/s within the beekeeping record book[c]
	Number of food comb removal events per colony/year	No	NA	Derived from other variable/s within the beekeeping record book[c]
Supplementary feeding Note: Detailed information on the composition of the supplementary feeding is not addressed by previous questionnaires	Date and amount of carbohydrates	Yes : Brodschneider and Crailsheim (2010); NBU questionnaire, 2014; COLOSS questionnaire, 2015 BeeNet	COLOSS asks for quantity of sugar feed; NBU provides a predefined list of additional feed types (and an additional open field for other types of feed) and it asks for feed quantity provided and month of application. Brodschneider and Crailsheim (2010) asks for date and type of carbohydrate feedingBeeNet asks for type of feed provided (open field) and its month of application.	Derived from other variable/s within the beekeeping record book[c]

Factor	Variable	Used in previous studies	Notes	Target (beekeeper, inspector, or it can be derived from other variable/s within the beekeeping record book[(c)])
	Date and quantity of proteins (including essential amino acids quantity if known)	Yes: BeeNet NBU	BeeNet asks for type of feed provided (open field) and its month of application.NBU provides a predefined list of additional feed types (and an additional open field for other types of feed) and it asks for feed quantity provided and month of application.	Derived from other variable/s within the beekeeping record book[(c)]
	Date and quantity of lipids	Yes:BeeNet NBU	BeeNet asks for type of feed provided (open field) and its month of application.NBU provides a predefined list of additional feed types (and an additional open field for other types of feed) and it asks for feed quantity provided and month of application.	Derived from other variable/s within the beekeeping record book[(c)]
	Date and duration of water supply	No	NA	Beekeeper
	Other bee feed:			
	• Date and quantity of micronutrients and vitamins (not important see below)	No	NA	Beekeeper
	• Date and quantity of probiotics (not important see below)	No	NA	Beekeeper

Factor	Variable	Used in previous studies	Notes	Target (beekeeper, inspector, or it can be derived from other variable/s within the beekeeping record book[(c)])
Production type of the colony	Honey [Y/N]	Yes: EPILOBEE, 2012–2014; NBU	NBU[(a)]	Beekeeper
	Pollen [Y/N]	Yes: EPILOBEE 2012–2014; NBU	NBU[(a)] does not have a specific option for this type of production, but does have an 'other' option with open field that can be filled	Beekeeper
	Bee packages (worker bees) [Y/N]	Yes: EPILOBEE 2012–2014; NBU	EPILOBEE and NBU[(a)] does not have a specific option for this type of production, but does have an 'other' option (NBU has an open field that can be filled).	Beekeeper
	Royal jelly [Y/N]	Yes: EPILOBEE 2012–2014; NBU	NBU[(a)] does not have a specific option for this type of production, but does have an 'other' option with open field that can be filled	Beekeeper
	Queens [Y/N]	Yes: EPILOBEE 2012–2014; NBU	NBU[(a)] EPILOBEE asks for n of queen produced by the beekeeper	Beekeeper
	Nucleus (queen, workers and brood) [Y/N]	Yes EPILOBEE 2012–2014; NBU	NBU[(a)]	Beekeeper
	Propolis [Y/N]	Yes EPILOBEE 2012–2014; NBU	EPILOBEE and NBU[(a)] does not have a specific option for this type of production, but does have an 'other' option (NBU has an open field that can be filled).	Beekeeper
	Wax [Y/N]	Yes EPILOBEE 2012–2014; NBU	EPILOBEE does not have a specific option for this type of production, but does have an 'other' option	Beekeeper

Factor	Variable	Used in previous studies	Notes	Target (beekeeper, inspector, or it can be derived from other variable/s within the beekeeping record book[c])
	Venom [Y/N]	Yes EPILOBEE 2012–2014; NBU	EPILOBEE and NBU[a] does not have a specific option for this type of production, but does have an 'other' option (NBU has an open field that can be filled)	Beekeeper
	Hive rental (compensated) [Y/N]	Yes EPILOBEE 2012–2014; NBU	NBU[a] does not have a specific option for this type of production, but does have an 'other' option (with open field to be filled	Beekeeper
Change in number of workers	Addition			
	• Number of events per colony/ year	No	NA	Derived from other variable/s within the beekeeping record book[c]
	• Quantity for each event 'weight or number'	No	NA	Beekeeper
	• Date of the event	No	NA	Beekeeper
	Origin of the workers for each event			
	• Same apiary	No	NA	Beekeeper
	• Different location (original coordinates)	No	NA	Beekeeper
	Removal			
	• Number of event s per colony/year	No	NA	Derived from other variable/s within the beekeeping record book[c]
	• Quantity for each event 'weight or number'	No	NA	Beekeeper
	• Date of each event	No	NA	Beekeeper

Factor	Variable	Used in previous studies	Notes	Target (beekeeper, inspector, or it can be derived from other variable/s within the beekeeping record book[(c)])
Introduction of a queen bee	Date of introduction/colony	Yes EPILOBEE 2012–2014	NA	Beekeeper
	Reason of introduction			
	• Original queen bee was intentionally removed (i.e. colony division) [Y/N]	No	NA	Beekeeper
	• Original queen bee was missing [Y/N]	No	NA	Beekeeper
	• Queen bee improvement [Y/N]	No	NA	Beekeeper
	Number of introduction events per colony/year	Yes EPILOBEE 2012–2014	NA	Beekeeper
	Introduction success			
	• The queen was accepted and successfully laid eggs afterwards [Y/N]	No	NA	Beekeeper
	• Rejection rate per colony/year	No	NA	Derived from other variable/s within the beekeeping record book[(c)]
	Geographic origin of the queen bee	Yes EPILOBEE 2012–2014	NA	Beekeeper
	Genetic origin of the queen bee	Yes EPILOBEE 2012–2014	EPILOBEE asks for the genetic origin of the colony (not specifically related to queen introduction)	Beekeeper (+laboratory analysis provider)
	Produced by the beekeeper within his/her apiary	No	NA	Beekeeper

Factor	Variable	Used in previous studies	Notes	Target (beekeeper, inspector, or it can be derived from other variable/s within the beekeeping record book[(c)])
Swarm control	Number of interventions per colony/year (swarm control effort)	No	NA	Derived from other variable/s within the beekeeping record book[(c)]
	Queen cell removal			
	• Date of first event	No	NA	Beekeeper
	• Number of cells per colony/ year	No	NA	Beekeeper
	Management of colony size			
	• Addition of empty frames (i.e. wax sheets) [Y/N]	No	NA	Beekeeper
	• Removal of brood and adult bees [Y/N]	No	NA	Beekeeper
Migration activity	Number of migration events per colony/year	Yes NBU questionnaire 2014; EPILOBEE 2012–2014; Van der Zee et al. (2013); COLOSS questionnaire 2015; US National Honey Bee Disease and Pest Survey	NA	Derived from other variable/s within the beekeeping record book[(c)]
	Date of migration event and distance between new and original apiary location.	Yes EPILOBEE 2012–2014	EPILOBEE asks for start and end date	Beekeeper
Location of the apiary	Location of the colony (coordinates)	Yes EPILOBEE 2012–2014, NBU questionnaire, US National Honey Bee Disease and Pest Survey questionnaire, BeeNet Project, 2013, COLOSS questionnaire 2015	NA	Beekeeper; some Member States have systems in place to register apiary locations (e.g. France, Italy). However, accessibility to these data is still to be assessed

Factor	Variable	Used in previous studies	Notes	Target (beekeeper, inspector, or it can be derived from other variable/s within the beekeeping record book[(c)])
	Duration at each GPS position	No	NA	Derived from other variable/s within the beekeeping record book[(c)]
	Location of the apiary	No	NA	Beekeeper
	Spatial distribution of the colonies within the apiary	No	NA	Beekeeper
	Average distance between the colonies of the same apiary	No	NA	Beekeeper
Size of the apiary	Total number of colonies	Yes NBU questionnaire 2014; EPILOBEE 2012–2014; US National Honey Bee Disease and Pest Survey; BeeNet	NA	Beekeeper
	Number of productive colonies	Yes NBU questionnaire 2014; COLOSS questionnaire 2015	NA	Beekeeper
	Change in number of colonies			
	Number of colonies added	Yes NBU questionnaire 2014; EPILOBEE 2012–2014	NA	Beekeeper
	Date of the event	No	NA	Beekeeper
	Addition rate per apiary/year	No	NA	Derived from other variable/s within the beekeeping record book[(c)]
	Origin of the colony			
	• Same apiary	N	NA	Beekeeper
	Artificial swarm [Y/N]	Yes EPILOBEE 2012–2014	EPILOBEE asks for number of colonies	Beekeeper
	Natural swarm [Y/N]	Yes EPILOBEE 2012–2014	EPILOBEE asks for number of colonies	Beekeeper

Factor	Variable	Used in previous studies	Notes	Target (beekeeper, inspector, or it can be derived from other variable/s within the beekeeping record book[(c)])
	Different location			
	• Original GPS coordinates of the introduced colony	No	NA	Beekeeper
	Removal (i.e. 'merge' if colonies are from the same apiary)	No	NA	Beekeeper
	Number of colonies removed	Yes NBU questionnaire 2014	NA	Beekeeper
	Removal rate /apiary/year	No	NA	Derived from other variable/s within the beekeeping record book[(c)]
	Dead colonies			
	• Number of dead colonies per year	Yes	COLOSS	Beekeeper
	• Date of colony death	No	NA	Beekeeper
Proximity of colonies belonging to other apiaries	Number of hives located within the double of the maximum foraging distance (2–5.5 km) and the surface of the shared foraging area	No	NA	Beekeeper (Some Member States have systems in place to register apiary locations as France and Italy. However, accessibility to these data is still to be assessed)

(a): NBU asks to rank the most commonly sold products (1 = most common).
(b): BeeNet recorded for each comb (present inside the colony) the presence of adult bees, brood, eggs, sealed honey and not sealed honey, pollen (four categories of abundance were used).
(c): Derived from beekeeper record book = general variables could be derived by assessing specific variables (e.g. 'Number of physical control method/year' can be estimated by summing individual events such as Varroa control methods with no chemicals used: worker brood removal [Y/N] + drone brood removal [Y/N] + queen confinement + heat treatment of brood/bees).

C.3. Colony outputs

Table C.32: Methods for measuring provisioning service – weighing the harvested honey

Indicator		**Assessing variable [unit]**	**Test method**
Harvested products		Quantity of harvested honey per year [kg/year]	Weighing the harvested honey
Test characteristics		**Information on the method of analysis**	
Protocol	Test objective	The quantity of honey harvested by the beekeeper during 1 year can be easily assessed by asking the beekeeper. If the assessment takes place before the honey-harvesting season, then supers can be weighed and the weight of the empty super subtracted. The mass difference is the quantity of honey in the super per hive. In cases where several supers are present in the hive, all the calculated masses should be summed, because the goal is to know the total quantity of honey per hive	
	Number of samples	All the supers of one hive	
	Matrix to be sampled	All the supers of one hive	
	Temporal and spatial aspects (sampling)	When the beekeeper harvests the honey (variable across Europe)	
Evaluation	Test sensitivity and LOD/LOQ	High because the honey is harvested and weighed	
	Test specificity	High. Wax removed for harvesting the honey is not considered to greatly influence final mass difference. The weight of bee bread or pollen, if present, should be deducted from that of honey. The evaluation of their weight should be done using the methods described for each constituent	
	Required time, equipment, expertise and availability/dissemination of method (sampling/analysis)	Required equipment: normal equipment for honey extraction and scale Normal activity for the beekeeper, so it does not represent extra time. No need to be an expert beekeeper to extract honey. The super contains mainly honey, but in some cases, the queen can also lay eggs in some part of the super (in the lower part). Then, it is more suitable to calculate the surfaces. When there is only honey in the super, it might be easier to weight it than to take photographs or to use grids. The difference between the weight of a full and empty super gives the amount of honey harvested	
	Standardisation	Standardised method. It is strongly recommended that super is marked with the number of the hive, and the super (with the empty combs) is weighed before being put in the hive	
	Possible limitations to use in field survey	No limitations. This activity is routinely performed by beekeepers	

LOD: limit of detection; LOQ: limit of quantification.

Table C.33: Methods for assessing regulating services – pollination service

Test method	Test characteristics	Notes
Measuring pollination demand		
Field assessments	The most holistic means to quantify the demands for pollination services is based on the number of visits required per RPU to provide optimal pollination services (in terms of economic output for crops or propagation of seeds in wild plants) (Liss et al., 2013). To date this method has not been formally applied. The number of visits required by a particular taxa (in this case, honeybees) to provide pollination services can be assessed in two ways: using cage studies or analysis of pollen deposition rates (Garratt et al., 2016).	Strengths Provides an accurate assessment of pollination service demands. • Can be used to quantify the change in visitation required to quantify pollination service deficits. • Only requires a single field season to generate data that can be used repeatedly and across all suitable habitats. Weaknesses • Very labour intensive and requires substantial expertise. • Requires substantial replication to provide an accurate estimation of pollen deposition and number of visits required.
Floral coverage	A more basic method for estimating demand is simply to estimate the number of floral units within a habitat that would benefit from pollination. Although this method is simpler than that above it does not provide an indication of the full extent of demand because different plants, particularly crops, may require substantially different visitation rates in order for optimal pollination to be achieved (e.g. Delaplane and Mayer, 2000; Winfree et al., 2011).	Strengths • Very simple to measure based on a single field survey. • Can be easily mapped and reapplied with new mapping data. Weaknesses • Does not measure the full extent of the demand.
Measuring pollination supply		
Field-based methods		
1) Plant sample selection	1) Sample units can be: a) Individual plants (requires data on plant density per RPU to extrapolate upwards but is easiest). b) Small plots (recommended for mass flowering crops e.g. oilseed rape; typically 1 × 1 m). c) Whole fields (almost impossible to undertake in most fields due to the high costs involved). 2) If selecting individual plants or plots then it is recommended that 12–20 sampling units are randomly selected within the field. Stratified random sampling can be used to take paired samples at different distances from the field edge or from hives placed in the field (this captures the effect of distance on pollination services, which typically declining further from hives). 3) If sampling individual plants of a tree crop, a single branch is an effective proxy for a whole plant yield (Vassiere et al., in review).	Measuring the supply of pollination services to plants within an RPU has two components. First, there should be a measure of the proportion of pollination services that are provided by honeybees. Second, there should be a measure of pollination service deficit. Pollination service deficit is the difference between supply and demand. This is evaluated as the difference between the observed levels of plant seed set/crop output (open) and the maximum possible levels of pollination (hand-pollination) assuming all other factors within the field are equal. This has three stages detailed below: To date there are a number of methods to assess the supply of pollination services from honeybees. This section overviews the three most relevant: (1) field-based assessments of actual pollen supplies and demands, (2) in-hive measures of supply with estimates of demand, and (3) modelling-based approaches. The following section outlines each of these methods including assessments of their relative strengths and weaknesses. Most of these methods are derived from Vassiere et al. (2011), Delaplane et al. (2013a,b) and Garratt et al. (2014).

Test method	Test characteristics	Notes
2) Sampling pollinator visitors to determine the proportion of visits by the focal taxa	1) On each sampling unit, a number of timed observations should be conducted to assess the proportion of honeybee crop visitors to the focal plant. 2) Observations should note the number of visitors and their approximate identity (this can simply be honeybee or non-honeybee visitors) and the proportion of legitimate pollinating (pollinators make contact with the sigma) and non-pollinating visits (pollinators do not contact the stigma) made by each taxa. 3) Observational transect walks in the surrounding habitat, including field margins are also recommended in case honeybees are visiting non-crop plants more than the crop itself. These should take the same format of times of visits to a particular floral patch, but there is less need to concern the observer with the legitimacy of visits. 4) Pollen traps placed on hives can also be used to collect pollen that can then be identified using light microscopy/genetic analysis, however, it should be noted that these will only show how important the plant is to the hive not how important the hives are to the plant (but see the section on pollination and honeybee health at the end of the document).	None
3) Evaluating pollination service deficits	1) Divide the sample in half (6–10); half of the sample will be open pollinated (marked but otherwise untouched) and half will be hand-pollinated. Hand-pollination represents the maximum level of pollination. 2) Plants are hand-pollinated via a paint brush using pollen either collected previously from died anthers or by simply transferring pollen from flower to flower. Fairly large amounts of pollen should be used to maximise pollination. Certain plants are not effectively pollinated by paint brushes (e.g. tomatoes) and may require other methods of artificial pollination. 3) Some species are self-incompatible (e.g. apples, some blueberries) and require pollen from another variety to pollinate them effectively. These pollinisers are usually planted in the crop field. 4) Once hand-pollinated, samples should be covered in 0.2 mm gauze to prevent excessive insect visitation from damaging the developing fruits.	Strengths • Very comprehensive, approximating the exact pollination service supply provided by local honeybees. • Can be used to identify economically relevant pollination service deficits to crops and potential risks to wild plant populations. • Provides an indication of the importance of honeybees within the wider visitor community. • Can be integrated into modelling based methods (see below). Weaknesses • Very costly, labour intensive and requires significant technical expertise. • Very demanding to assess multiple RPUs within 5 km. • Visitation rates may be skewed by forage preferences • Does not directly link services with specific hives.

Test method	Test characteristics	Notes
	5) Deficit should be based on the final seed set (for wild plants) or final harvested yield of crops (total weight of fruits/nuts/pods set) of each sample. Sampling only the initial fruit/seed set will ignore post-set abortion and, in the case of crops, any management practices (e.g. fruit thinning in fruit trees), however this can provide a back-up measure in case of high levels of pest damage to sample points. 6) For crops, ideally deficit should be based on the difference in profit from open and hand-pollinated samples as it is this factor that producers are interested in and represents the ultimate end of pollination services (Garratt et al., 2014). a) To estimate the differences in profit it is important to account for the differences in fruit quality (based on the EU's guidelines for class 1 or class 2 fruit) and any changes in management that may occur (e.g. increased fruit thinning costs from a higher proportion of fruit set). These will vary between crops and should be considered throughout. b) Profit differences are the differences in total market prices for a crop minus the difference in the affected variable costs of producing it (it is not necessary to account for all costs as many of these are not dependent upon crop output). 1) If the differences between the treatments are ~ 0 or negative then there is no pollination service deficit, if the differences are notable and positive then there is a service deficit, indicating that supply does not match demand. 2) If the number of pollen grains required to supply demands, the change in the number of visits (positive or negative) required to optimise pollination services can be estimated.	
4) In-hive pollen collection	Because honeybee pollination is indicative of a healthy hive, a simpler method to assess service supply is to estimate the net amount of pollen that a honeybee collects per visit (using the methods described in Section 3.4.2). Using pollen traps and dissection of in hive pollen (also described in Section 3.4.2) it will be possible to estimate the number of visits that workers from a hive are making to different plants within the surrounding landscape. The effectiveness of honeybees as pollinators can be assessed using field observations (described above).	Strengths • Requires minimal effort and expertise to collect raw data because pollen can be sampled passively using pollen traps and actively from the hive with relative ease. • Provides an estimate of exactly what the hive is providing pollination services to. • Can be combined with existing estimates of resource use as part of a wider protocol. • Can use data pooled from beekeepers across the Union, evaluated at a central repository.

Test method	Test characteristics	Notes
		Weaknesses • Pollen of any species can be very cryptic to identify, requiring substantial time and expertise to identify samples. • Requires substantial preparatory work to assess the amount of pollen collected per visit. • Needs to be combined with expert heavy field work to identify if honeybees are effective pollinators. • Some pollen will be lost in transit, making it difficult to assess accurately how much pollen was collected. • May require complex modelling with other work to estimate whether colonies are supplying demands. Does not indicate where pollen has come from, making it difficult to identify where services are being supplied to
Modelling methods		
	Statistical models can be used to generate large-scale maps of pollination service potential across a landscape based on the position of the hive and the relative abundance and attractiveness of pollen and nectar resources within the wider landscape. The most comprehensive of such models developed thus far is Lonsdorf et al. (2009), which has been adapted numerous times by other studies (but see Schulp et al., 2014a). To date, this model has only been applied to wild pollinators, however, it can potentially be applied to honeybees as well. These models require four principle sources of data: 1) Spatially explicit maps of honeybees within a landscape (e.g. national registers of honeybee hives). 2) Spatially explicit maps of the surrounding landscape (e.g. CORINE or other land use maps, such as CEH's Land Cover map in the UK). 3) Estimates of the quantity of pollen and nectar resources provided within a habitat (ideally collected from field data as per Section 3.4.2). 4) Estimates of the attractiveness of these resources to honeybees.	Strengths • Requires no field data to undertake. • Can be estimated for large areas simultaneously. • Can be used to project changes in pollination services following landscape alterations. • Can be continually refined and revised using new field data. Weaknesses • Spatially explicit maps of honeybees are unlikely to be available in many countries. • Habitat maps are often lacking in specific details (e.g. which crops are grown in arable fields). • Professional beekeepers often move their hives, making a single census per year unrealistic. • Using expert opinion is likely to lead to significant estimation error. • Attractiveness is hard to quantify. • Does not indicate colony health as no measures of actual service are taken.

Test method	Test characteristics	Notes
	The first two items are used to generate the spatial data on which the models are able to predict the foraging habits of the hives. The availability of suitable data sets, however, is likely to vary substantially between the Member States. Although items 3 and 4 can be derived from observational field studies, expert opinion can instead be used if it is collected in a systematic and rigorous manner. Field observations of honeybee visitation and efficiency (described above) can be used to determine fruit weight and validate the model. Information linking honeybee health to pollination services remains largely speculative at this point, however, any effects of health on foraging (e.g. colony fecundity, foraging habits when healthy/ unhealthy), and therefore pollination services, could be incorporated into models and synchronised with data collected on the health of individual colonies, allowing for more accurate forecasting of supplies.	Existing data sets There are no suitable data sets currently available that apply any of these methods to provide estimates of pollination services across the EU.

Appendix D – Clinical signs of disease

Some clinical signs, for instance the crawling of bees, can be caused by infectious agents (Morse and Flottum, 1997; Ribière et al., 2010), pests (Harrison et al., 2001) and/or intoxication (Johansen, 1977; Gregorc, 2012). Therefore, it is important to carry out a differential diagnosis.

Table D.1: Examples of signs of disease frequently observed in adult bees and brood, and in the hive[(a)]

Inspection of	Clinical signs of disease – some examples
Adult bee population	Weakening or reduced colony population Collapsed colony/empty hives
Hive	Traces of diarrhoea outside and/or within the hive Galleries inside the combs Abnormal behaviour at the flight board
Brood	Mosaic brood/spotty brood pattern Capping with small holes Abnormal sealing of cells Presence of mites in brood cells Light brown to brown dead larvae Ropey larvae Neglected/slumped/discoloured/cannibalised brood Dried dead larvae Scale Deformed/dead pupae Dead honeybees with deformed wings in sealed cells Dead emerging bees in brood with extended proboscis Cannibalism of larvae or pupae Mite faeces Brood destruction

(a): Some of these clinical signs may also be due to pesticide poisoning.

Table D.2: Clinical signs compatible with *Varroa destructor*, *Tropilaelaps* spp., *Aethina tumida*, *Paenibacillus larvae*, *Melissococcus plutonius*, *Nosema* spp., ABPV, CBPV, DWV, SBV and *Vespa velutina* infection or infestation

Agent	Disease clinical signs	Case definition for confirmation
Varroa destructor	**Colony** Reduced colony population and/or altered demography Collapsed colony / empty hives **Adult honeybees** Presence of *Varroa* mites on the honeybees above a threshold (phoretic *Varroa*) Smaller bees Crawling bees Honeybees with deformed and/or atrophied wings Honeybees with a small abdomen **Brood** Mosaic brood/ spotty brood pattern Capping with small hole(s) (abnormal perforations in caps) Abnormal sealing of cells Presence of *Varroa* mites in brood cells (brown mature females, white immature stages) Light brown to brown dead larvae (absence of the AFB ropey aspect) Neglected/slumped/discoloured/cannibalised brood Dried dead larvae Deformed/dead pupae Dead honeybees with deformed wings in sealed cells Dead emerging bees in brood with extended proboscis (only the head emerges, with the tongue sticking out) Cannibalism of larvae or pupae Mite faeces	A colony is considered positive for varroosis if typical clinical signs have been recorded in the field and/or if any laboratory analysis has diagnosed the disease
Tropilaelaps spp.	Clinical signs of infestation with *Tropilaelaps* mites are similar those of varroosis clinical signs. However, it should be remembered that those clinical signs occur at a late stage in the infestation. Therefore, it is crucial to detect atypical mites. This disease is currently exotic to the EU Deformed wing honeybees Deformed abdomen Capping with small hole Irregular brood (spotty brood pattern) Dead brood	Identification of *Tropilaelaps* spp. at national level and preferably confirmation by the EU reference laboratory
Aethina tumida (small hive beetle)	Observation of phoretic beetle, larvae or eggs Galleries inside the combs Brood destruction Modification of the honey colour and honey fermentation	Identification of *Aethina tumida* at national level and preferably confirmation by the EU reference laboratory

Agent	Disease clinical signs	Case definition for confirmation
Paenibacillus larvae (AFB)	Spotty' brood pattern/mosaic brood Capping with different colour/dark sunken cell capping/holes in cappings Ropey larvae (match test) Coffee brown coloured larva Tongue of dead larvae pointing upwards Sticky scales Specific odour of sick larvae	Any colony presenting clinical signs and infected with *Paenibacillus larvae* is considered suffering from American foulbrood
Melissococcus plutonius (EFB)	Mosaic brood/spotty brood pattern Capping with holes Slumped larva Larvae with a yellowish to brown colour (generally in unsealed brood) Vinegar or putrefaction odour of the larvae	Any colony presenting clinical signs and infested with *Melissococcus plutonius* is considered suffering from European foulbrood
Nosema spp.	Dead honeybees in front of the hive (not agent-specific) Crawling honeybees, bees clinging to the grass (not agent-specific) Traces of diarrhoea outside and/or within the hive Reduction of the colony population and/or altered demography	A colony is considered positive with nosemosis if typical clinical signs (see disease form) have been recorded and *Nosema* spp. spores have been detected
ABPV	Brood and adult mortalities Presence of *Varroa destructor*	
CBPV	Trembling honeybees, crawling honeybees Dead honeybees in front of the hive Small black honeybees, shiny and hairless rejected from the hive Honeybees rejected by guards Abnormal behaviour at the flight board Bees with bloated abdomens, sometimes 'diarrhoea'	Any colony presenting clinical signs and infected with CBPV at viral load superior to 8 Log copies of the viral genome per honeybee is considered to be suffering from chronic paralysis. Taking into account the uncertainty of the measurement (U = 2.06 Log copies per bee) estimated by an interlaboratory proficiency test (EURL, unpublished data), a virus load result between 6 and 8 Log copies per bee should nevertheless be indicative of a potential chronic paralysis case
DWV	Brood and adult mortalities Vestigial and crumpled wings Bloated abdomens Severely shortened adult life span for emerging worker and drone bees Presence of *Varroa destructor*	
SBV	Discoloured, sunken or perforated cappings, pupa present with undeveloped head Larvae sit in the cell with their heads raised Skin of the larvae that becomes a fluid filled sac Pale yellow larvae becoming dark brown from the anterior end	

Agent	Disease clinical signs	Case definition for confirmation
Vespa velutina[(a)]	The presence of Vv flying (e.g. from middle July to around October) Regarding the presence of bees on the flight board, there are two contrasting cases: (i) bees are numerous on the flight board and on the front wall of the hive, or (ii) there no bees on the flight board but the bees are inside the colony and the opening to the hive is full of bees in order to prevent hornets entering the hive The foraging activity could be very weak Presence of hornets hovering in front of the hive (sometimes inside the colony) Change in the return flight to the hive (zigzag, when the number of hornets is still low)	

ABPV: acute bee paralysis virus; AFB: American foulbrood; CBPV: chronic bee paralysis virus; DWV: deformed wing virus; SBV: sacbrood virus.
(a): *Vespa velutina* is included in the mind map 'Resource providing unit' because this is a predator of *Apis mellifera* and its impact on honeybee health in the European areas where it is present is high.

Appendix E – Contaminants in bee products

Table E.1: Contaminants in bee products[(c)]

Group	LOD/LOQ [µg/kg] (ppb)	Examples of compounds of the group
Amide	21.3/42.7[(a)]	Benalaxyl
	4.9/14.8[(a)]	Prochloraz
Avermectine	nd/nd[(a)]	Abamectin
	nd/nd[(a)]	Eprinomectin
	nd/nd[(a)]	Ivermectin
	nd/nd[(a)]	Moxidectin
Carbamate	5.0/10.0[(b)]	Adicarb
	5.0/10.0[(b)]	Adicarb sulfon
	5.0/10.0[(b)]	Adicarb sulfoxide
	0.7/1.2[(a)]	Carbaryl
	5.0/10.0[(b)]	Carbaryl
	0.1/1.0[(a)]	Carbendazim
	0.4/1.0[(a)]	Carbofuran
	5.0/10.0[(b)]	Carbofuran
	0.6/1.9[(a)]	Diethofencarb
	20.3/28.4[(a)]	Fenarimol
	1.0/3.3[(a)]	Fenoxycarb
	5.0/10.0[(b)]	Mercaptodimethur
	5.0/10.0[(b)]	Mercaptodimethur sulfon
	5.0/10.0[(b)]	Mercaptodimethur sulfoxide
	0.2/0.5[(a)]	Methiocarb
	0.8/3.2[(a)]	Methomyl
	5.0/10.0[(b)]	Methomyl
	5.0/10.0[(b)]	Oxamyl
	16.5/51.5[(a)]	Thiophanate-methyl
Insect growth regulator	2.1/8.6[(a)]	Pyriproxifen IGR
	29.9/59.9[(a)]	Buprofezin IGR
Neonicotinoid	1.4/17.0[(a)]	Clothianidin
	2.6/12.0[(a)], 0.2/1.0[(b)]	Imidacloprid
	2.0/8.5[(a)]	Thiamethoxam
	0.2/0.6[(b)]	6-chloronicotinic acid
Organohalogen	11.1/13.9[(a)]	Aldrin
	1.0/14.5[(a)]	Bromopropylate
	11.1/22.2[(a)]	Chlorothalonil
	4.6/13.9[(a)]	DDD o,p'
	11.0/27.4[(a)]	DDT p,p'
	47.5/nd[(a)]	Dicloran
	9.8/24.6[(a)]	Dieldrin
	0.1/8.0[(b)]	Endosulfan
	12.7/31.7[(a)]	Endosulfan alpha
	15.5/51.5[(a)]	Endosulfan beta
	8.4/21.1[(a)]	Endosulfan sulfate
	8.6/17.2[(a)], 0.1/4.0[(b)]	Lindane
	9.7/24.3[(a)]	Hexachlorobenzene
	2.0/9.8[(a)]	Methoxychlor
	8.2/20.4[(a)]	Tetradifon

Group	LOD/LOQ [µg/kg] (ppb)	Examples of compounds of the group
Organophosphorus	57.0/196.7[(b)]	Azinphos-methyl
	8.9/22.3[(a)]	Cadusaphos
	8.0/20.0[(a)]	Chlorpyriphos
	10.0/34.5[(b)]	Chlorpyriphos
	15.6/52.0 (LC)[(a)]	Chlorpyriphos-methyl
	1.3/19.5 (GC)[(a)]	Chlorpyriphos-methyl
	1.8/6.0 (LC)[(a)]	Coumaphos
	4.6/18.4 (GC)[(a)]	Coumaphos
	37.0/142.6[(b)]	Coumaphos
	10.5/26.3[(a)]	Diazinon
	14.6/21.9[(a)]	Dichlorvos
	9.1/45.4[(a)]	Dimethoate
	18.0/59.6[(b)]	Dimethoate
	3.2/13.7[(a)]	Ethoprofos
	3.9/19.4[(a)]	Fenitrothion
	19.0/66.9[(b)]	Fenitrothion
	8.0/30.6[(b)]	Fenthion
	39.1/58.6[(a)]	Malathion
	9.0/31.5[(b)]	Malathion
	2.2/25.1[(a)]	Methamidophos
	13.0/49.6[(b)]	Methidathion
	3.8/27.7[(b)]	Mevinphos
	11.4/17.1[(a)]	Parathion
	8.0/30.4[(b)]	Parathion ethyl
	10.0/39.5[(b)]	Parathion methyl
	1.4/14.4 (1)	Phenthoate
	10.2/15.4 (1)	Phosalone
	14.8/24.6 (1)	Phosmet
	2.7/15.5 (1)	Phoxim
	1.1/11.4 (1)	Tolclofos-methyl
	0.5/9.3 (1)	Triphenylphosphate
Phenylpyrazole/Pyrazole	0.3/0.5[(b)]	Fipronil
	0.3/0.5[(b)]	Fipronil desulfinyl
	0.3/0.5[(b)]	Fipronil sulfon
Pyrimidine	2.8/21.4[(a)]	Bupirimate
	20.3/28.4[(a)]	Fenarimol
Pyrethroid	4.5/19.3[(a)]	Bifenthrin
	76.9/230.7[(a)]	Cyfluthrin
	7.0/98.7[(b)]	Cyfluthrin
	56.4/169.1[(a)]	Cypermethrin
	3.8/93.3[(b)]	Cypermethrin
	28.9/57.8[(a)]	Deltamethrin
	0.1/29.9[(b)]	Deltamethrin
	25.1/150.9[(a)]	Esfenvalerate
	5.3/32.1[(a)]	Permethrin
	4.6/22.8[(a)]	Tau-fluvalinate
	1.1/76.0[(b)]	Tau-fluvalinate
	23.9/47.9[(a)]	λ cyhalothrin
Dicarboximide	nd/nd[(a)]	Procymidone
	1.5/12.6[(a)]	Vinclozolin
	15.6/48.7[(a)]	Iprodione

Group	LOD/LOQ [μg/kg] (ppb)	Examples of compounds of the group
Formamidine	46.3/69.4[(a)]	Amitraz I
	8.1/17.3[(a)]	Amitraz II
Imidazole	6.9/25.5[(a)]	Imazalil
Tetrazine	9.7/48.6[(a)]	Clofentezine
Thiazolidine	4.8/10.2[(a)]	Hexythiazox
Triazole	3.9/16.5[(a)]	Bitertanol
	10.1/50.4 (GC)[(a)]	Cyproconazole
	3.0/10.1 (LC)[(a)]	Cyproconazole
	5.0/10.0[(b)]	Cyproconazole
	5.0/10.0[(b)]	Epoxyconazole
	3.6/15.5[(a)]	Flusilazole
	5.0/10.0[(b)]	Flusilazole
	5.0/20.0[(b)]	Hexaconazole
	10.7/37.5[(a)]	Myclobutanil
	5.0/10.0[(b)]	Myclobutanil
	4.3/85.1[(a)]	Propiconazole
	5.0/10.0[(b)]	Propiconazole
	6.7/16.9[(a)]	Penconazole
	5.0/10.0[(b)]	Penconazole
	12.8/38.4[(a)]	Tebuconazole
	10.0/20.0[(b)]	Tebuconazole
	5.0/10.0[(b)]	Tetraconazole
	5.6/19.2 (LC)[(a)]	Triadimenol
	16.0/32.0 (GC)[(a)]	Triadimenol
	3.8/10.8[(a)]	Paclobutrazide
Synergist	9.0/45.2 (GC)[(a)]	Piperonyl Butoxide
	6.8/22.6 (LC)[(a)]	Piperonyl Butoxide

GC: gas chromatography; GC–MS: gas chromatography–mass spectrometry; GC-ToF: gas chromatography time-of-flight; LC: liquid chromatography; LC–ESI-MS: liquid chromatography–electrospray ionisation-mass spectrometry; LC–MS/MS: liquid chromatography with tandem mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; MS/MS: tandem mass spectrometry; QuEChERS: Quick, Easy, Cheap, Effective, Rugged and Safe.
(a): Wiest et al. (2011), pollen loads. Extraction, QuEChERS (ng/g); analyses, GC-ToF and LC–MS/MS.
(b): Chauzat et al. (2011), pollen loads. LC–MS/MS.
(c): See also EFSA, 2012a,b, table G.1.

Supporting information to Table E.1

LOD and LOQ values for some substances can also be found in the publications listed below:

- Bee bread: The final extract was analysed by GC–MS and LC–MS/MS for 258 pesticides and pesticide metabolites. The LOQ values were between 3 and 10 μg/kg, and in a few cases 15 μg/kg. For all neonicotinoids the LOD values were at the level of 1 μg/kg. Genersch (2010)
- Pollen pellets. All samples were analysed using a modified version of the QuEChERS protocol. MS/MS. LOD (ppb): thiamethoxam, 0.5; clothianidin, 1.0; azoxystrobin, 0.5; trifloxystrobin, 0.4; propiconazole, 2.0. Krupke et al. (2012)
- Seven neonicotinoid insecticides (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam) were analysed in pollen. Once the neonicotinoids were extracted, they were determined using an optimised LC–ESI-MS method, which was validated in terms of selectivity, linearity, precision and recovery. The LOD and LOQ values were 0.4–2.8 μg/kg and 1.2–9.1 μg/kg, respectively. Cepero et al. (2014a)
- High-performance liquid chromatography with mass spectrophotometric detection (HPLC–MS/MS). The LOQ of the method was 1 mg/kg for both thiamethoxam and CGA322704 in bee pollen and bee bread stored. Pilling et al. (2013).

Appendix F – Worker behaviour catalogue

Table F.1: Honeybee worker behavioural catalogue (taken from Scheiner et al., 2013)

Task	Description
Cell cleaning	Removing debris from used brood cells (cocoons, larvae excretion), cleaning cell walls. Takes place in a cell not currently being used
General nest sanitation	Removing debris from nest (mouldy pollen, old cappings, dead brood, and dead adults)
Brood care	Feeding larvae (head in brood cell > 1.3 min), attending queen
Construction	Smoothing wooden hive parts with mandibles and manipulating wax and propolis in cracks and corners of the hive
Fanning wings	Flapping wings while standing in hive/at entrance
Food care	Insertion of head into a cell containing nectar, receiving nectar-on bridge
Grooming a nestmate	Running nestmate body parts through mandibles
Grooming	Self-running own body parts through mandibles
Inspecting	A cell momentary insertion of the anterior portion of the head into an empty cell
Nest care	Manipulating wax of cells (not cappings), building new empty cells
Patrolling	Walking around nest
Standing and chaining	Standing stationary or hanging while stationary on nestmates
Brood cap manipulation	Trimming or smoothing wax cappings on brood cells and capping brood with wax
Honey cap manipulation	Trimming or smoothing wax cappings on cells of honey and capping honey with wax
Trophallaxis	Nestmate exchange of food (not near entrance), receiver thrusts tongue at donators mouthpart, donator opens mouthparts pushes tongue forward, and regurgitates a drop which is lapped up
Vibrating	Fast rhythmic body vibrations (non-dance)
Head in pollen	Insertion of head into a cell containing pollen
Inspecting	Brood head in brood cell, < 1.3 min
Dancing	Dancing without/with pollen
Washboarding/plaining	Standing and rocking back and forth with mouthparts open
Attending dance	Dance attendance without/with pollen

Appendix G – Protocol for data collection by the beekeeper on indicators scored as H-HH

Area of interest	Details of the area	Indicator (H-HH)	Test method	Method description	Time needed	Timing
2 m around the hive	–	Presence of dead bees	Visual inspection (presence/absence)	Table C.11	2–4 min	After winter, beekeeping season, before winter
		Atypical behaviour	Visual inspection (presence/absence)	Table C.23		After winter, beekeeping season, before winter
		Predators (e.g. *Vespa velutina*)	Visual inspection (presence/absence)	–		After winter, beekeeping season, before winter
Hive entrance (outside)	–	Presence of dead bees	Visual inspection (presence/absence)	Table C.11	1–2 min	After winter, beekeeping season, before winter
		Atypical behaviour	Visual inspection (presence/absence)	Table C.23		After winter, beekeeping season, before winter
		Foraging activity	Foraging rate [number of foraging bees per minute in the morning; number of foraging bees per minute in the early afternoon]	Table C.21	10 min in the morning + 10 min in the afternoon	After winter, beekeeping season, before winter
Inside the hive	Above the combs	Queen presence	Visual inspection. Presence and identification (is it a new queen?)	Tables C.1, C.2, C.3		After winter, beekeeping season, before winter
	Both sides of all combs	Queen presence/queen natural replacement/ queen longevity	Visual inspection. Presence and identification of the queen (if not found previously)	Tables C.1, C.2, C.3	20–30 min	After winter, beekeeping season, before winter
	Combs	Clinical signs in adult bees and brood	Visual inspection	Table C.24, Appendix D		After winter, beekeeping season, before winter
		Atypical behaviour	Visual inspection of atypical behaviour	Table C.22		After winter, beekeeping season, before winter
		Colony size	Visual estimation	Table C.4		After winter, beekeeping season, before winter
		Queen laying viable eggs	Visual identification of the presence of eggs, larvae and pupae	Table C.2		After winter, beekeeping season, before winter

Area of interest	Details of the area	Indicator (H-HH)	Test method	Method description	Time needed	Timing
		Brood, bee bread	Visual estimation of the surface of the combs containing honey, bee bread, brood and covered by adult bees	Tables C.3, C.13		After winter, beekeeping season, before winter
	Bottom of the hive	Presence of dead bees	Visual estimation	Table C.11		After winter, beekeeping season, before winter
	Sampling	*Varroa*	Sampling of bees, alcohol washing test, counting *Varroa*	Table C.26	5 min	Before winter
	Sampling	American foulbrood	Sampling of a piece of comb with larvae	Table C.27	10–20 min	Before winter
		Bee bread pesticide contamination	Sampling of bee bread; 1–2 times per year	Table C.19		After winter, beekeeping season, before winter
		Wax pesticide contamination	Sampling of wax; 1–2 times per year	Table C.20		After winter, beekeeping season, before winter
		Harvested honey	Weighing of the super/ weight of harvested honey	Table C.32		After beekeeping season

Appendix H – Analysis of bee health

Summary statistics from a stratified sampling design weights information according to the relative frequency of design classes. It is possible to develop aggregation models depending on the purpose of the analysis and the information available. Such upscaling is possible using statistical (Snijders and Bosker, 2012; Gelman et al., 2014) and geostatistical aggregation methods (Müller et al., 1997; Wackernagel, 2003). The latter refers to analysis of spatiotemporal data, in which there is an underlying, often spatially continuous, model (also known as a random field) the output of which depends on the (spatial) location of data, e.g. a colony. Two useful interpolation techniques are Kriging (e.g. in Chilès and Delfiner, 1999) and Gaussian random fields (Cameletti et al., 2013).

Statistical modelling is useful for considering differences in health between sampling design strata, or between spatial units when aggregating. For example, when scaling up from a colony to an administrative (e.g. NUTS 3) level, possible differences in health status associated with beekeeping practices within the NUTS 3 region can be taken into account by weighting the aggregated bee health measure depending on the relative frequency of different beekeeping practices in the NUTS 3 region of interest. Further, interpolation between colony data may consider statistical associations between health and ecoclimatic variables. At the end, both ecoclimatic relevant categories (e.g. major land use defined by CORINE level 3) and other drivers (e.g. beekeeping practice or disease infestations or exposure to pesticides) can be considered when aggregating data collected at colony levels.

When describing the HSI at levels higher than the colony itself, it is important to allow for estimates of variation (variability and uncertainty) in HSI. For example, given the relative frequencies of strata variables, the relative frequencies of HSI can be described, in addition to an average HSI.

It is possible to aggregate data or predictions from a lower to a higher level (e.g. from colony to apiary), but not the other way around. Thus, collecting and analysing data is recommended at colony level and is also compatible with generating outputs at higher levels. However, there are exceptions where data can be collected at apiary level if the information can be applied to all colonies of the apiary (e.g. beekeeper characteristics).

Analysis models with high predictive ability are better at detecting signals of deterioration in health and separate these from random noise and errors. It is therefore important to use validated and well-supported mechanistic models that make use of all the data collected.

Statistical analyses of health status may differ in complexity depending on the extent to which they define health status using indicators that can be directly observed (observables[35]) or that are latent variables emerging from an analysis of multiple indicators. A latent variable can be seen as an index of colony health status. Health status of a colony defined by observables must be more than one indicator of interest to provide a holistic description of health.

Statistical analyses may further differ into the extent to which they take account of causal relations between factors and indicators. Analyses range from modelling of statistical associations to explicit modelling of causal relations. The modelling of causal relations can use more or less complex mechanistic models, which describe our understanding of process and system dynamics.

By combining these choices, different approaches to statistical modelling can be attained. For example, binomial regression of winter mortality on a set of predictors in a cross-sectional study (Van der Zee et al., 2015) exemplifies models assessing health status from relative frequencies obtained in field measurements (i.e. something observable). Such regressions result in estimated statistical associations or, if preceded by a casual graph, causalities. Causalities may be valid for the state of a colony over time. For example, a good predictor of the state of colony may be the previous state of the colony at last inspection. The previous state in combination with other factors can produce a process model of colony dynamics, which takes into account time to help prediction of health based on observed colony attributes within a season. Approaches that define health from multiple attributes and indicators, without taking one as the primary characteristic of health, can be based on non-causal or causal modelling. To exemplify these aspects, four approaches to analyse and model bee health are elaborated:

- approach 1 – quantify bee health as a latent variable from multivariate analysis;
- approach 2 – classify bee health in a colony using a decision tree based on the colony attributes;
- approach 3 – predict bee health by causal modelling;
- approach 4 – predict bee health by process-based modelling.

[35] Variables that can be measured.

These approaches are related to each other and can overlap (Figure H.1). Approaches 1 and 2 represent alternative ways to define an HSI in a way that the assessment is based on more than one indicator. The third and fourth approaches describe ways to link factors to health and to model changes in health. With the exception of approach 2, it is possible to address more than one analytical goal with an approach for analysis production (Table H.1).

Table H.1: An overview of the four analysis approaches provided and to what extent they fall within the four analysis goals

	Analytical goal			
Analysis production approach	**Descriptive**	**Explanatory**	**Predictive**	**Prescriptive**
1. Quantify bee health as an emerging latent variable	x	x	x	
2. Classify bee health	x			
3. Causal modelling of bee health		x	x	x
4. Process-based modelling of bee health		x	x	x

These four approaches can provide added value for beekeepers and scientists. A validated classification model (approach 2) can help a beekeeper to assess health status based on their own observations of the colony attributes. It is possible to integrate the classification model into an influence diagram, which shows the impacts of management conditional on environmental drivers. The influence diagram can be turned into a network model, which when trained and validated based on data collected in a harmonised way, can be turned into a tested decision support system.

All the approaches described here aid in the identification of key drivers of bee health and may use the colony as the unit of interest. These are all quantitative analyses that allow uncertainty to be accounted for under predictive and prescriptive analysis goals. Variability (aleatory uncertainty), such as random variation between colonies, apiaries and regions, and within and between years, can be estimated from ranges of outcomes (e.g. approach 1) or be expressed explicitly in the model (e.g. approach 4). With a characterisation of variability, it is possible to detect early signs of deterioration in managed honeybee health when the pattern starts to deviate from what is expected or considered normal.

The latent variables from approach 1 and predictions of bee health in approaches 3 and 4 (possibly integrated with the classification in approach 2) can efficiently detect patterns and anomalies in multivariate data. Even though an HSI aims to describe health, it can be easier for decision makers, beekeepers and scientists to understand estimates and predictions of physically meaningful parameters, the latter usually variables that possible to observe (see Approach 1).

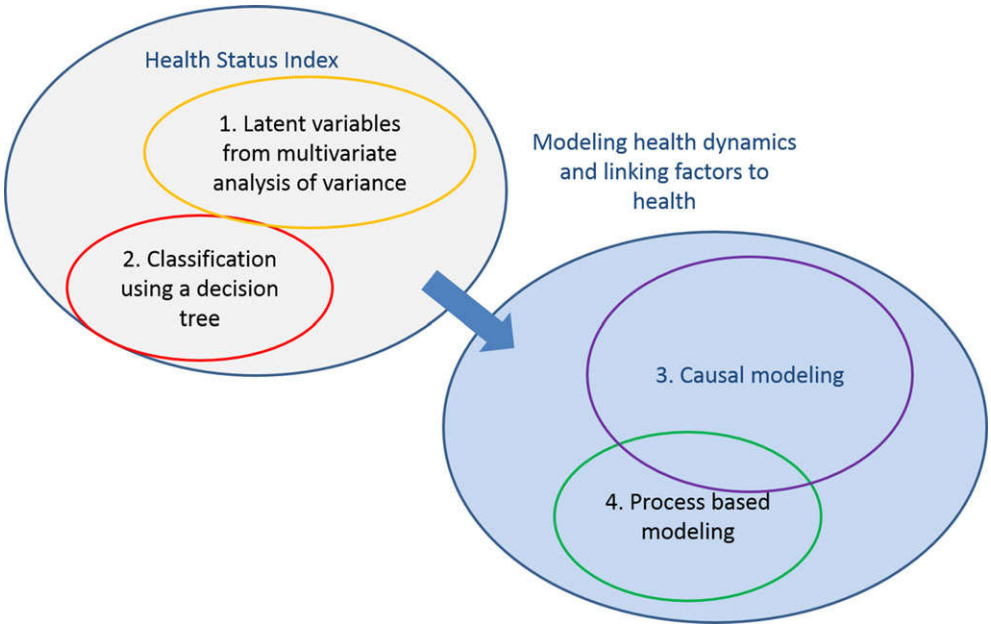


Figure H.1: The relation and possible overlap between the four approaches to model bee health

Approach 1 – Quantify bee health as a latent variable from multivariate analysis of variance

Description of the main characteristics and properties of multivariate analysis

Since honeybee health depends on many factors and can be appraised by many quantitative indicators, we define it as a multivariate problem. Hence, it may be addressed naturally by multivariate analysis of variance. Multivariate analysis of variance forms a family of statistical techniques aiming at describing how several variables jointly vary. Multivariate analysis is based on linear combinations of variables. These linear combinations can be explained as geometrical projections of data to axes in a lower dimensional space. An analysis of variance seeks parameter values to construct these linear combinations of variables and parameters to relate these linear combinations to each other. The linear combinations are alternatively interpreted as latent variables representing system properties or phenomena, which cannot be measured directly. Instead, it is assumed that it is possible to identify and study these latent variables by studying the joint variation in observations of variables that depend upon them. Thus, multivariate analysis does not attempt to explicitly describe any processes underlying variation in the data or model stochastic behaviour. A general reference to multivariate analysis of variance is, for instance, in Legendre and Legendre (2012), while a more in-depth description can be found, for instance, in Anderson, 1958; Mardia et al., 1979.

An advantage of multivariate analysis is the potential to represent graphically how a group of variables vary jointly based on the underlying data. Geometrical projections can efficiently illustrate potential linear relations in data and the variance explained by these. Because multivariate analysis of variance model linear relationships, these methods may fail to detect possible non-linearities in data.

Descriptive multivariate analysis of variance seeks to find projections that capture variance in a multivariate data set, or alternatively, the projections that best discriminate between clusters in the data. Two types of explanatory and predictive multivariate analysis of variance are possible. The first uses the linear combinations explaining a substantial proportion of variance in the data (i.e. relevant latent variables) as response variable(s) in explanatory or predictive analyses. For example, the first principal components from a principal component analysis can be used as a response in a regression analysis with covariates or predictors, which is then referred to as principal component regression (Hastie et al., 2009). The second type of predictive analysis is to generate projections (or latent variables) while considering the impacts of covariates or predictors. For example, the partial least squares technique (Wold, 1982) derives latent variables in both predictors and responses, such that it jointly maximises the variation explained in both data sets and the covariation between them. The second type of multivariate analysis is an example of supervised statistical learning, for which there are plenty of algorithms and machine learning techniques (Hastie et al., 2009).

Many methods for multivariate analysis of variance are able to deal with different types of problems and readily available tools exist in open source software. The partial least squares technique has, for example, been developed to consider mixtures of continuous, ordinal and categorical data (Esposito Vinzi et al., 2010). This releases a critical limitation of the original principal component analysis or partial least square regression, which use only continuous data. There is also the possibility to create blocks within the data sets or to introduce a hierarchy in the relations between latent variables. For example, partial least squares path modelling (Tenenhaus et al., 2005) combines multivariate analysis of variance with causal modelling (see more details under approach 3, Appendix H).

Some applications and examples for its use in analysing bee health

Multivariate analysis of variance can be applied to the analysis of bee health based on data collected in accordance with the recommendations in TOR3. Hypothesis testing in explanatory analyses with several response variables occasionally tests the hypothesis in question (such as the influence of a driver) on each response separately (Jacques et al., 2016; APENET reported by Porrini et al., 2016). This may reduce the power of each statistical test, which can then be accounted for (Shaffer, 1995). Multivariate analysis of variance (MANOVA) is a common analysis for hypothesis testing when there are more than one response variable (e.g. Cutler et al., 2014).

In the context of bee health, we are interested in multivariate analysis applied on indicators to describe and predict health. It is possible to describe health using the latent variables emerging from a multivariate analysis on indicators (Figure H.2A). These latent variables may represent an inherent property of a colony, e.g. a bee HSI, or be used as response variables in explanatory and predictive analyses. The HSI will be a linear combination of indicators. A descriptive latent variable of health may be

derived based on a multivariate analysis of the indicator using, for example, principal component analysis, latent class analysis, factor analysis or discriminant analysis. An example of application of this approach is hierarchical clustering applied to some of the bee health indicators suggested here in order to derive a new response variable in the analysis of the EPILOBEE data (Jacques et al., 2016). However, it is important that the new response variable or the HSI is constructed such that it is a latent variable which best discriminates bad from good health conditions based on colony attributes or colony outputs. Note that latent variables primarily explains variation in multivariate data and not automatically know what constitutes good or bad health. There is, therefore, no guarantee that the latent variables represent gradients of health and the parameters in the model must be studied carefully.

Multivariate analysis with the aim of making predictions needs to take into account at least two data sets, the covariates and the responses. Here, covariates (or predictors, in case of a predictive analysis goal) are taken from the drivers (i.e. variables related to BMP, RPU and environmental drivers) and responses are taken from the attributes of bee health. Thus, for the analyses carried out to assess bee health, it is likely that responses and covariates both are multivariate. In manner similar to that for the indicators, it is possible to apply multivariate analysis to the drivers with the purpose of seeing how these covary and even use the emerging latent variables as gradients of stressors (Figure H.2B). However, the added value of carrying out an analysis on drivers before knowing the importance of these on bee health is small. A joint analysis of variance of indicators and drivers, e.g. using the partial least square method, may identify latent variables based on the drivers which are able to predict latent variables based on the indicators. It is even possible to graphically illustrate the relation between drivers and indicators in one graph (Figure H.2C). Because the covariates are assumed to cause the responses, a predictive multivariate analysis is actually an example of causal modelling (as described in approach 3, Appendix H). Recent model developments, like the mixtures of multivariate analysis and causal modelling, such as partial least squares path modelling (Tenenhaus et al., 2005), may overcome the limitations of a pure multivariate analysis and increase the usefulness in risk assessment. A PLS path analysis may start with assuming casual relations between drivers and indicators and between indicators and colony outputs (Figure H.3). The inclusion of causal structures removes parameters from the multivariate analysis (by assigning them a value of zero) when deriving the latent variables. The results can be presented using causal graphs showing the relative importance and direction of changes in the individual variables within a set (Figure H.4).

Given a validated predictive model, the latent variables explain variation and the model can be used to identify changes in the latent variables from year to year. The latent variables for the drivers may be used as a stressor index for bee health, which can be monitored separately from the health index. Ranges of variability in the HSI and stressor index can be assessed from the data. It is recommended to verify the predictive performance and reliability of models generated from multivariate analysis techniques, e.g. by testing them on new data or by means of cross-validation (Hastie et al., 2009). When the number of variables is large, these methods rely on large sample sizes to produce reliable predictive models and ranges of variability. Trends and the detection of anomalies in bee health can be identified by statistical process control by, for example, control charts (see Benneyan et al., 2003 for an introduction related to health care management). Risk managers may want to monitor the HSI to follow impacts of policy or regulation. Monitoring can make use of control charts for the HSI and individual indicators may raise an alert when patterns deviate from normal.

A validated predictive multivariate analysis can be used to target which variables (indicators and factors) to measure in the field to efficiently predict the impact of changes in drivers (covariates). This might result in leaner field surveys in the future and in the identification of indicators and drivers linked to early detection of deterioration in colony health. However, confidence in multivariate methods depends on the quality and quantity of the data available. Removing a factor or indicator at an early stage of monitoring may result in a disproportional loss of information. This is a critical issue for systems with high inherent variability, such as bee health.

Elaborated example multivariate analysis of variance

Here, we constructed an artificial data set of attributes – queen, disease, products, behaviour and demography – and of drivers – beekeeping management, resource providing unit and environmental drivers.

First, we perform a multivariate analysis on the attributes, namely a principal component analysis (PCA). The artificial attributes are continuous variables with no missing data. PCA is a method to project data onto axes of a lower dimension, also known as the principal components. These components can also be seen as latent variables. The principal components are ordered by the amount

of variance in data that is explained by them. The PCA is able to identify two principal components that explain 59% and 36% of the variance in attributes, respectively. If we use the first component as a health status indicator, we must be sure that it reflects a gradient from poor to good health. This can be done by studying parameters (loadings) to construct the principal components.

Having a multivariate set of predictors, we could carry out a PCA on the drivers as well. The principal components in Figure H.2B show the pattern in drivers in the collected data. There is nothing in the figure saying which drivers are important and how they influence health.

A predictive multivariate analysis method is partial least squares (PLS) regression. A PLS carried out on the same data on attributes and drivers results in a completely new set of latent variables. There are two types of PLS components, one for the attributes (brown) and one for the drivers (blue). Laid on top of each other (Figure H.2C) one can see how they covary, i.e. which drivers have the strongest influence on which attributes. This is a very efficient way to illustrate the covariation between two multivariate data. However, there is still no guarantee that the first PLS component for the attributes shows a gradient from poor to good health.

PLS analysis can be extended to include more than two multivariate data sets using, for example, causal analysis in PLS path analysis. A PLS path analysis begins by defining the casual links (Figure H.3). Note that causal links are assigned from the beginning. If these are inaccurate the model will be inaccurate. The causal graph describes the relation between the latent variables (PLS components) in the model. The next step is to derive the PLS components, which are linear combinations of the variables in the corresponding data set. A more detailed description of the method and how to deal with data of different types is found in Tenenhaus et al. (2005). The contribution of each variable and the sign of its contribution are illustrated as separate diagrams (Figure H.4). It is possible to identify more advanced covariation between variables than is shown here.

The R-code for these examples can be downloaded from github.com/Ullrika/Healthy-B.

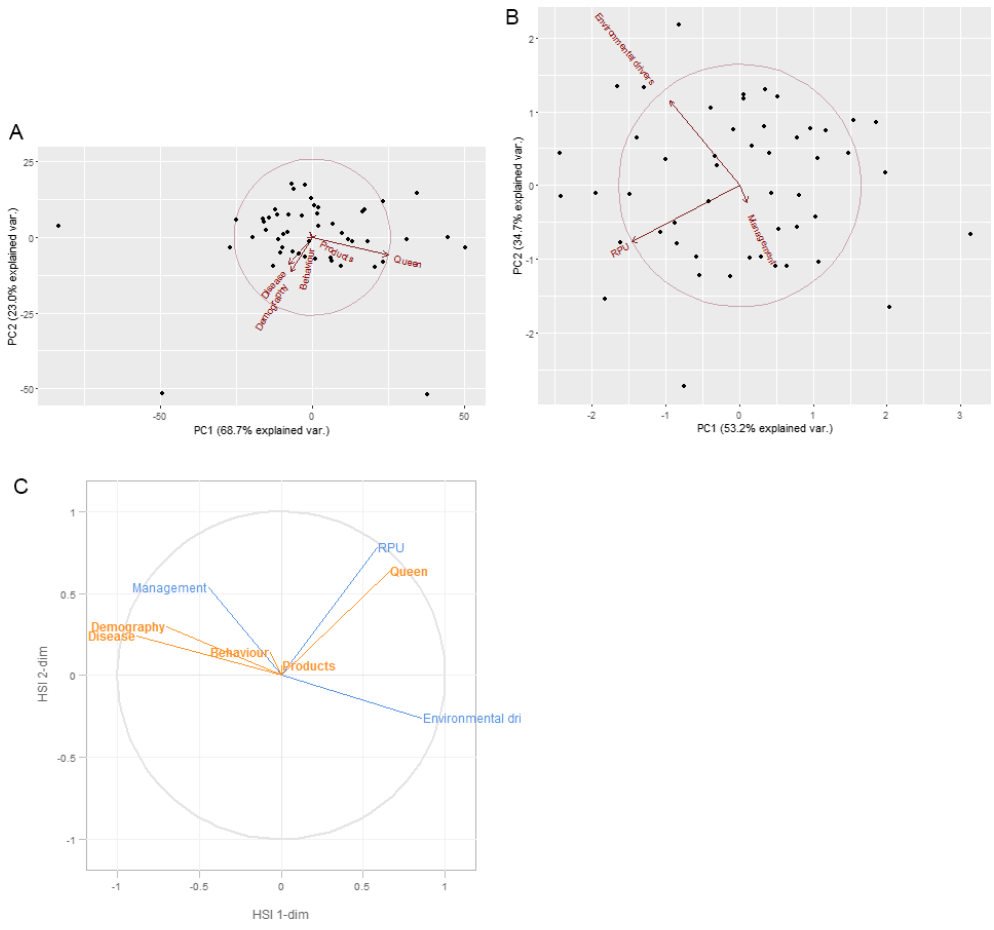


Figure H.2: Principal component analysis of (A) artificial attributes and (B) drivers. (C) A partial least squares regression on the same data

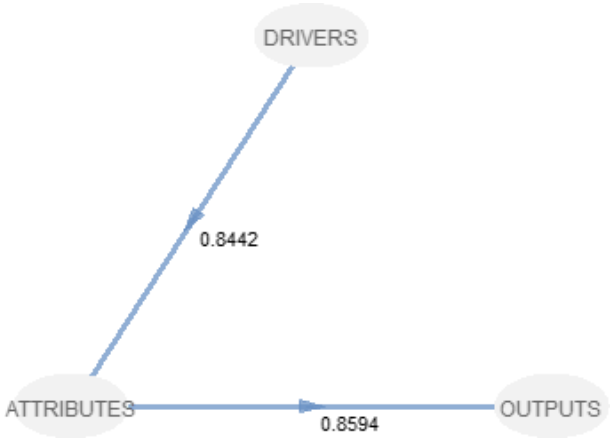


Figure H.3: A causal graph of the relation between drivers, attributes and colony outputs used in a partial least square path analysis. Each node is a multivariate data set and the colour and number of the arrows show the sign and strength of the casual link

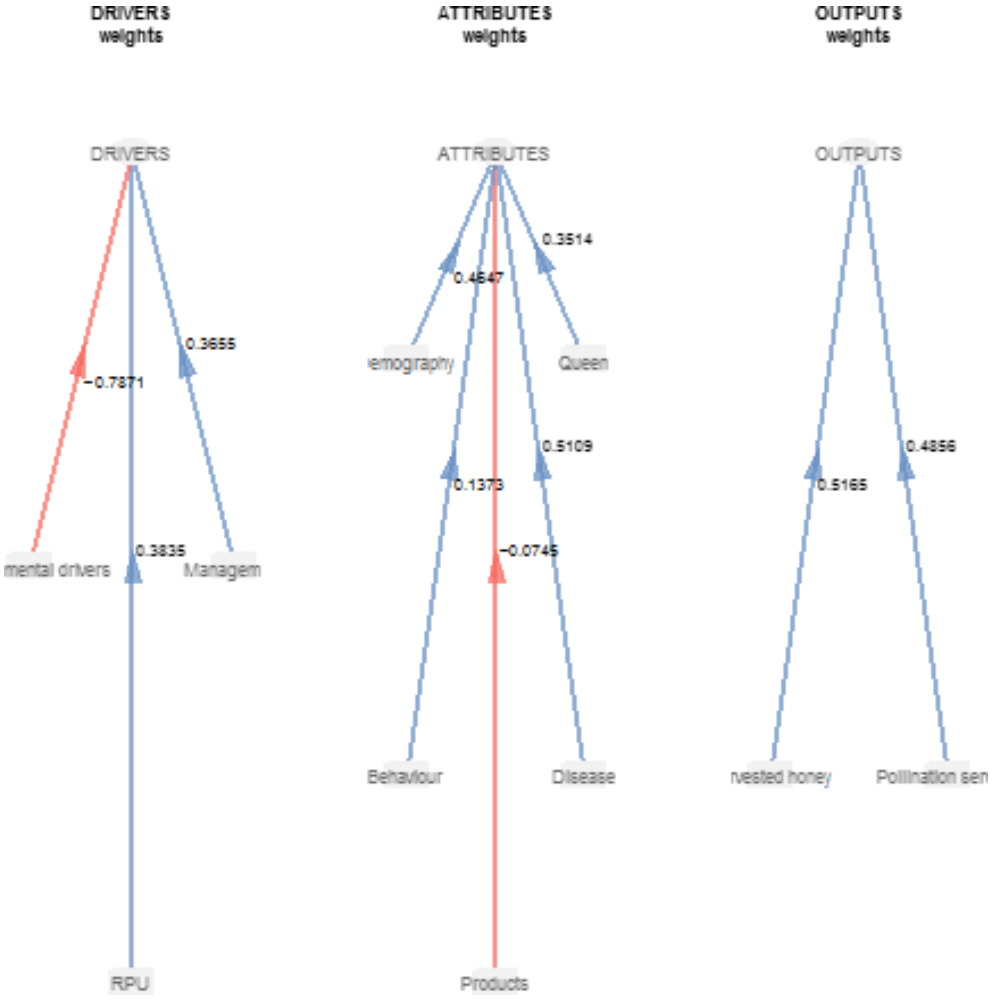


Figure H.4: The contribution of variables in an artificial multivariate data to the PLS components in a PLS path analysis. The sign shows the direction of change and the absolute number show the relative influence from a variable to the node in the causal graph in Figure H.7

Pro/cons of using multivariate analysis to assess bee health

Multivariate analyses are able to base the assessment of bee health on more than one health indicator and can use the colony as the unit of interest.

The latent variables emerging from a multivariate analysis are useful for detecting changes in patters, but may fail to describe health, because there is no guarantee that the direction of change in the HSI corresponds to an improvement or deterioration of health.

Because most multivariate analyses aim to describe and capture signals in variation and covariation, a limitation of these methods is that they are based on patterns and signals in data and do not include other types of information, such as theoretical models or expert knowledge. Furthermore, these methods do not handle non-linear relations or random errors in data, or variability in system dynamics or random system processes.

Multivariate analysis requires large data samples to discriminate between patterns and random noise, especially in a system where variability is high. Even though these analyses do not quantify variability explicitly, given enough data sampled under varying conditions, it is possible to use statistical methods to estimate ranges of variation and detect the early signs of a deterioration in managed honeybee health. Multivariate analysis is able to quantify uncertainty in output, mostly by resampling methods.

One advantage of multivariate analysis is that it can graphically illustrate high dimensional data without any advanced theory. A disadvantage is that components, such as latent variables and loadings can be difficult to interpret for both scientists and stakeholders. The multivariate methods are sensitive to scaling (i.e. standardisation or normalisation of variables to similar ranges), and careful consideration is required when data are of different types (e.g. continuous, nominal or ordinal data). Temporal scales may be captured by introducing causal modelling, for example, dependency over time. Spatial scales can be considered by introducing site-specific categorical variables, for example, NUT3 level, into the analysis.

Approach 2 – Classify bee health based on colony attributes using a decision tree

Description of the main characteristics and properties of decision trees

Event trees, fault trees and decision trees are examples of logical models with a wide use in risk assessment (Bedford and Cooke, 2001). A decision tree uses Boolean logical (such as AND, OR, NOT operators) to answer a question (e.g. what to do) based on observed events or states of a system. A decision tree can be used for classification or as an influence diagram showing the consequences of alternative decisions. Here, we focus on an approach to classify the health of a colony based on observations of colony attributes, and perhaps including pollination services. The aim is to find a classification taking multiple attributes into account at the same time and the possible interactions between them. An overview of decision tree models for classification can be found in Safavian and Landgreb (1991) and Bedford and Cooke (2001).

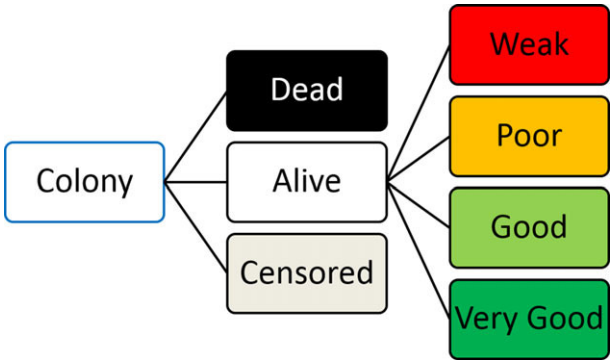
Techniques for classification using trees are either expert based or data driven. It is possible to train and test a statistical decision tree (e.g. regression trees – Hastie et al., 2009) using data for which health classes are known. In an analysis of bee health, there is no classification of the health status of a honeybee colony to use as a reference. The approach must therefore be to construct a classification of health, a categorical HSI, based on logical rules and assumed dependencies between colony attributes.

Some applications and examples of decision tree use in analysing bee health

An example of the implementation of decision trees in the context of bee health is the 'smart bee hive b+WSN' (Edwards-Murphy et al., 2016). They classify the health status of a bee hive (note, not of the colony) according to its temperature, humidity and CO_2 concentration with the aim of triggering alarms when deviations from normal conditions or ranges of these variables are prevailing. This paper compares a data-driven (threshold algorithm) and an expert-driven (machine learning decision tree algorithm) approach to building a decision tree. As expected, the data-driven approach has higher accuracy than the expert-driven decision tree. However, Edwards-Murphy et al. had access to high quality data sets on the judged health status of hives, which is a necessary condition when training a decision tree. They show in their work how a decision tree using microclimate variables recorded in hives can assist beekeepers in decision making. Here we are interested in a decision tree based on the colony indicators identified in TOR1–3.

Decision tree analysis can be used to define the HSI. This is described in detail in Appendix H and is summarised briefly here. The first step is to define what a colony is. Here, a colony is associated with a specific queen. In this scientific opinion, a new colony is created when a queen is replaced, by the beekeeper, by natural replacement or when it leaves the hive via a swarm (see Section 3.1.1). Therefore, a new colony is created whenever another queen replaces the current queen. Queen mortality results in the death of a colony; however, the worker bees and larvae can be used to build a new colony. Because queen replacement is common for honeybees, the classification model sees a colony as dead, alive or censored (e.g. when the queen is deliberately replaced by the beekeeper or naturally replaced by the workers) (Figure H.5).

Health refers to a colony that is alive (Figure H.5). However, colony death is a clear indication of bad health. The status 'censored by replacement' does not necessarily indicate health status, but will



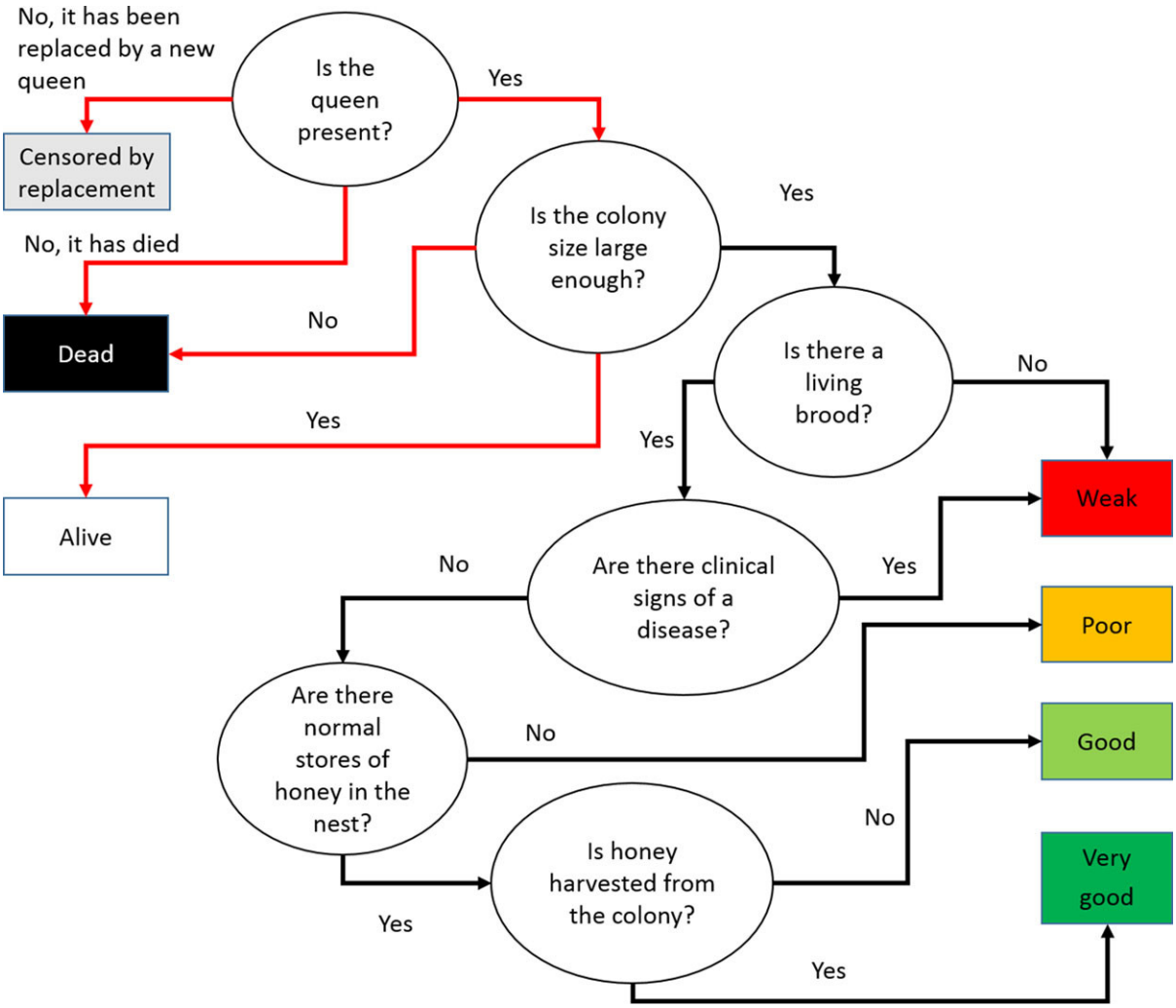
A colony can also be either dead or censored by replacement (e.g. when the queen is deliberately replaced by the beekeeper or naturally replaced by the workers).

Figure H.5: The structure behind classifying the health status of a colony. Health categories from weak to very good apply to a colony that is alive

influence the statistical analysis as they should for example not be included in the denominator in a calculation of mortality rates. The colony state 'censored' makes sure that the defined categories of health status cover all possible transitions between other colony states and health states.

The second step is to define what health categories a living colony can have. For example, if alive, the health of a colony could range from weak to very good (Figure H.5). How to distinguish weak from poor is fundamental in order to proceed to the next step.

The third step is to specify what status of colony and health should be assigned given the observed attributes and pollination service. What constitutes a good or poor health status of a colony that is alive for the different indicators of honeybee health identified in TORs 1–3 must now be specified in an operational manner. In Appendix H, a scheme is presented that specifies classification rules for each of the attributes demography, behaviour, disease, in-hive production and colony output pollination service. This scheme allows for local specific auxiliary variables taking into account that the characteristics of a healthy bee colony normally vary between eco-climatic regions. A simplified version of this decision tree is shown in Figure H.6. The red part of the decision tree classifies the colony state, whereas the black part is the decision tree and classifies the health state if the colony is alive. In reality, the classification considers multiple attributes jointly when assigning a health class, i.e. a colony suffering from *Varroa* infestation can still be classified as in 'good' health if it produces normal honey in the nest and for harvest.



What is a large size and normal stores of honey depend on when an inspection is made.

Figure H.6: A simplified version of a decision tree to classify health status of a bee colony seen as a combination of the colony state (alive, dead or censored) and health if alive (weak, poor, good and very good)

The health status of a colony is dynamic and could change over time due to changes in attributes, factors and drivers. An analysis seeks to link factors that explain or predict changes in health status or colony states. Colony mortality is a change in colony status. The health status of a colony changing from very good to poor is also worth studying and can bring added value when understanding health. Changes in types of health response (e.g. colony mortality or health status when alive) can be studied because it is recommended that a colony is inspected at least three times during a year in the context of a field survey (see Section 2.2.2). This means that data will, to some extent, be a colony-specific time-series (longitudinal) which allows for an analysis of changes in health status on a within-year time scale. The decision tree in Appendix H could be modified to jointly consider data collected at all three inspections during a year.

Experts can be uncertain about interactions between attributes and how to combine them to form different health status categories. A classification of bee health based on colony attributes and colony outputs can result in one health state per colony, but it is also possible to quantify uncertainty in a classification. Uncertainty can be considered by assigning probabilities to the branches in the tree, reflecting either the possibility of randomness in what health class to follow from a specific set of conditions (aleatory uncertainty) or a lack of knowledge of which health class to assign given a specific set of conditions (epistemic uncertainty). Bayesian belief networks (Pearl, 1995; Landuyt et al., 2013) are models that use probability to quantify uncertainty in linkages between the nodes in a network.

A decision tree is a type of causal model (see approach 3, Appendix H) and can be modelled as a Bayesian network. Probabilities on linkages between nodes are then expressed as conditional probability tables (Figure H.9). When there are many possible interactions between classification attributes, this uncertainty can propagate through the network, with a large impact on the classification.

Missing data or data errors can be a source of uncertainty as well. A decision tree can handle uncertainty in an observation, for example, uncertainty in clinical signs of a disease or errors in measurements of the level of *Varroa* infestation. Uncertainty in data is treated by propagating uncertainty in data (input) through the decision tree, resulting in uncertainty in the HSI classification (output).

Elaborated example bee health classification using a logical decision tree

Here, we have started to develop a classification of health using a decision tree. This is not a finalised model and needs to be modified further before being taken into use. The aim here is to demonstrate what a decision tree for the purpose to classification without any reference data on health may be like.

The classification model classifies the health status of a colony using a health status index with the classes: dead, weak, poor, good, very good or replaced. The health status index is generated based on two state-variables, namely the colony states – alive (white), dead (black) or replaced (grey) (Figure H.7) – and four levels of health state (from red to dark green) (see Table H.2).

Table H.2: Colony information, colony state and health status considered in the HSI

Colony information	ID
	Replacing colony ID
	Replaced by colony ID
Colony state (S)	Dead
	Alive
	Replaced
Health status (if alive) (H)	Weak
	Poor
	Good
	Very good

This division is required to follow changes in health. For colony with ID1 in Figure H.7A, the health state at time t, H_t, is 'Good', whereas health at the next inspection $t + 1$, H_{t+1}, is 'Poor'. The colony goes from alive to dead before the next inspection at $t + 2$. Statistical analysis may take into account that observations H_t and H_{t+1} for colony ID are dependent.

The second example (Figure H.7B) shows colony ID2 observed with a 'Very good' health state at time t. At the next inspection at $t + 1$, the colony has been replaced by colony ID3. In this case, the replacement was made by the beekeeper to split a good colony into two and was not triggered by the health status (which was 'Very good'). Even though this is a new colony, there may be dependencies between the health status of the colonies (dashed line), because they may share the same genetic material, wax and bee bread. It is therefore important to trace the colony ID and the faith of colonies as much as possible.

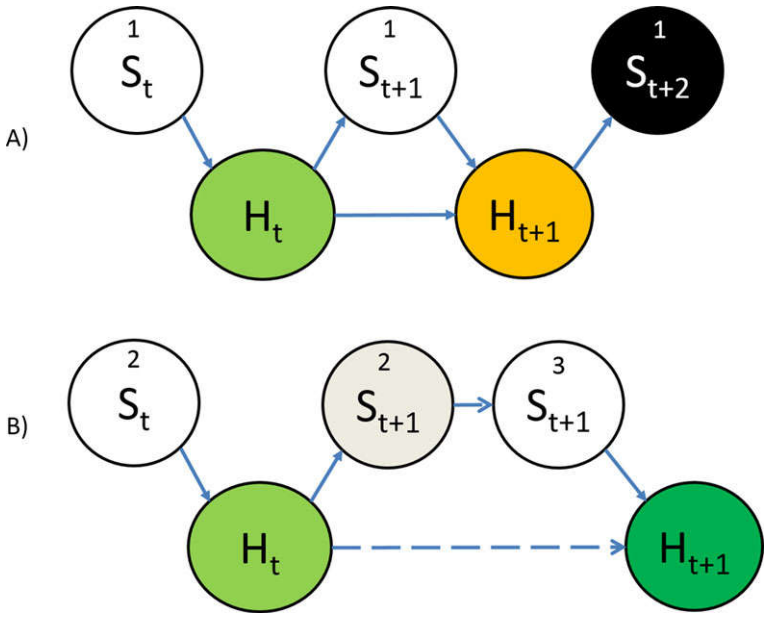


Figure H.7: Two examples of development of the state of a colony showing the situations that can appear in a statistical analysis

The classification model helps to trace colony ID and possible linkages to other colonies, together with health status. Intermediate state variables were included to aid classification: intermediate colony state (ICS) (Dead, Quasi-dead, Alive, Censored by swarming or replacement); intermediate health state, signs of weakening W (No signs, Indications, Clear signs); in-hive production P (None, Low, High); and colony outputs CO (Honey harvested, Pollination service providers). The attributes are linked to the intermediate variables or directly to the colony or health status. Figure H.8 shows the model as a simplified network.

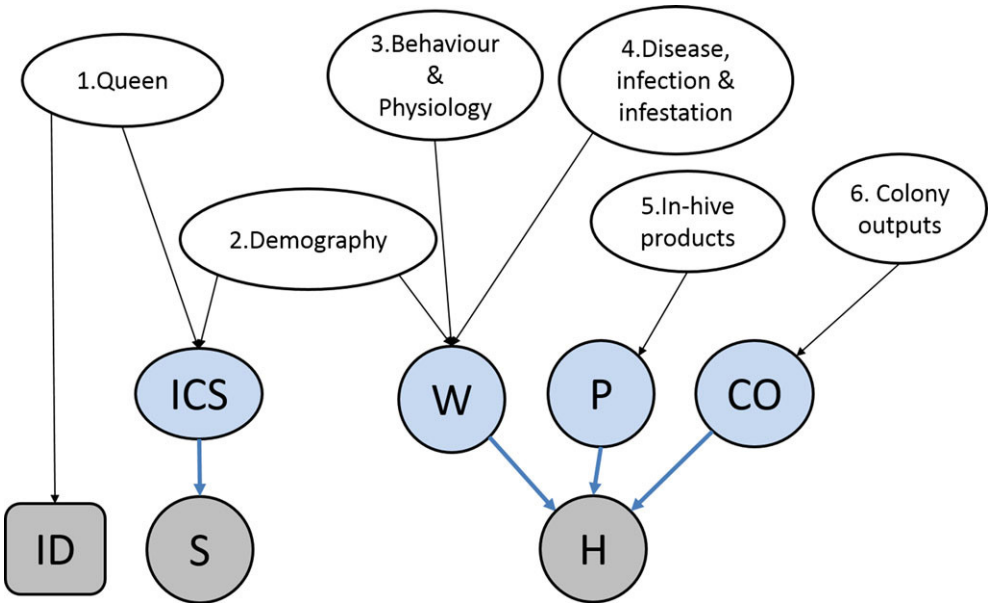


Figure H.8: See text for description

The health status of a honeybee colony is classified by answering Yes or No to the questions given in Table H.3, assigning values to intermediate variables according to the procedure in the table. The last step is to derive the final classification of health status based on the intermediate state variables (Table H.4 and Table H.5). The intermediate variables are integrated by Boolean logic (Figure H.6) or by conditional probability tables (Figure H.9). The latter uses probabilities to quantify uncertainty in

the associations between intermediate states and final status, and to propagate uncertainty in the answers to questions through the model to the final health status index.

Table H.3: The stepwise questions to assign intermediate colony state and intermediate health variables. This is a deliberately simplified decision tree with the purpose to illustrate the approach

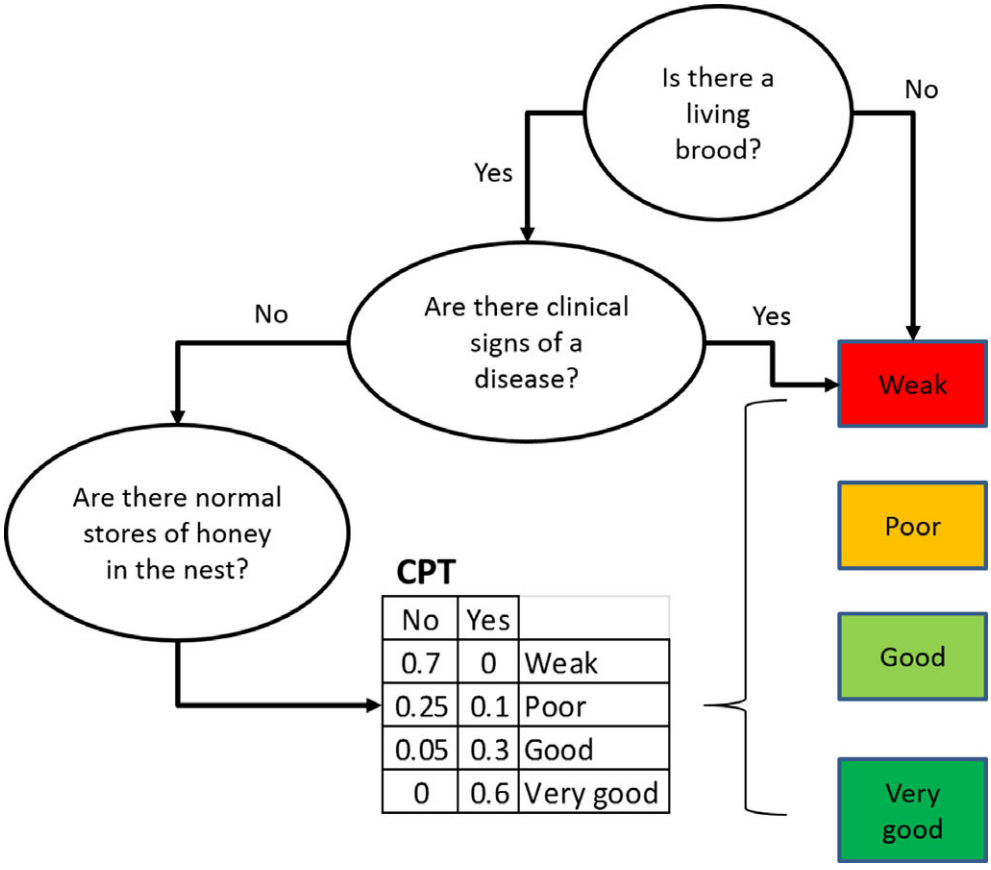
Step	Action/question	If answer is Yes	If answer is No
	Initialise		
	ICS = Alive		
	W = No signs		
1	Queen		
1.1	Has the colony been inspected before?	Go to 1.2	Start a new colony ID and go to 1.2
1.2.	Is a queen present?	If 1.1=yes, go to 1.3	ICS = Quasi-dead go to 2
1.3	Is it the old queen?	Go to 2	Go to 1.4
1.4	Has the queen been replaced by the beekeeper?	ICS = replaced, and start a new colony ID	Go to 1.5
1.5	Has the queen left with a swarm?	ICS = replaced, and start a new colony ID	Go to 2
2	Demography		
2.1	Is the colony population of large enough in relation to the geographical location of the apiary and the time in the year?	Go to 2.2	ICS = Quasi-dead, go to 2.2
2.2	Are there many dead bees?	W = Indications, go to 2.3	Go to 2.3
2.3	Is there a living brood?	Go to 3	W = Indications, go to 3
3	Behaviour and physiology		
3.1	Does the colony show atypical behaviour?	W = Indications, go to 4	Go to 4
4	Disease, infection and infestation		
4.1	Are there any clinical signs of infection?	W = Clear signs, go to 4.2	Go to 4.2
4.2	Are there signs of *Paenibacillus* larvae?	W = Clear signs, go to 4.3	Go to 4.3
4.3	Is *Varroa* present?	W = max (W, Indications), go to 4.4	Go to 4.4
4.4	Is *Varroa* infestation at high levels?	W = Clear signs, go to 5	Go to 5
5	In-hive products		
5.1	Are there stores of honey to be used by bees in the nest?	P = Low, go to 5.2	P = None, go to 5.3
5.2	Is there a normal production of honey in the super?	P = High, go to 5.3	P = Low, go to 5.3
5.3	Is there bee bread in the hive?	P = max(P, Low), go to 5.4	P = max(P, None), go to 5.4
5.4	Is there a normal amount of bee bread?	P = max(P, High), go to 5.5	P = max(P, Low), go to 5.5
6	Colony outputs		
6.1	Has honey been harvested from the colony?	Ho = Honey harvested	Ho = No honey harvested
6.2	Are the foragers of the colony providing pollination services?	PS = Service providers	PS = Not service providers

Table H.4: The rules to derive colony state given intermediate colony state

Colony state	Intermediate colony state
S	**ICS**
Dead	Dead or Quasi-dead
Alive	Alive
Censored by swarming or replacement	Censored by swarming or replacement

Table H.5: The rules to derive health state (H) given states of intermediate health variables (W, P) and colony outputs (Ho and PS)

Health Status	Signs of weakening	In hive production	Honey harvested	Pollination service
H	W	P	Ho	PS
Weak	Clear signs	None	No honey harvested	Not service providers
Poor	No signs OR Indications	Low	Honey harvested	Service providers
Good	No signs	Low OR High	Honey harvested	Service providers
Very good	No signs	High	Honey harvested	Service providers



Note that only a few attributes are shown in this tree.

Figure H.9: Turning the decision tree into a Bayesian belief network by adding conditional probability tables (CPT) on the links between nodes

Pro/cons of using decision trees to assess bee health

A decision tree is a structured way to classify bee health using the colony as the unit of interest, and is based on several colony attributes, when there is no possibility to train a model (i.e. to learn)

from data. The decision tree described in Appendix H gives an example of how to describe bee health at the colony level.

A decision tree is able to handle uncertainty in data and expert knowledge.

The health status classifications from an acceptable and expert-proven decision tree can be used as 'data' of a response variable in further analyses e.g. modelling the impact of external drivers on bee health. The HSI derived from a decision tree classification make it possible to detect early signs of deterioration by studying changes in the HSI of each observed colony in a descriptive analysis or forecasting in predictive analysis.

The decision tree in Appendix H demonstrates what a decision tree for HSI could be like, but must be developed further before it is ready to support a HSI for colony bee health.

A decision tree for an HSI based on collected data of colony attributes can be expanded to include more variables and can be integrated with other models that, for example, consider bee health at different temporal and spatial scales. Integrating the decision tree with explanatory or predictive analyses (e.g. approaches 3 and 4, see Appendix H) will be valuable because the HSI provides a holistic measure of bee health required to perform these analyses. Expanding the decision tree with management variables lays the basis for a decision support tool for beekeepers.

Approach 3 – Predict bee health by causal modelling

Description of the main characteristics and properties of causal modelling

'Causal modelling' is a well-known field in statistical and computer science (e.g. Koller and Friedman, 2009; Pearl, 2009; Hernán and Robins, 2016). Special cases of causal models include 'Bayesian networks' (Pearl, 1985; Neapolitan, 1989), 'log linear path models' (Hagenaars, 1993) and 'structural equation modelling' (Bollen, 1989). Structural equation modelling, in particular, may be the best-known of these techniques in systems biology and ecology (e.g. Shipley, 2000; Grace et al., 2010).

Various software packages exist to specify causal models and estimate their parameters from data, such as Mplus, Tetrad, the gR suite of R packages, R package lavaan, openMx (open source) and the Excel add-in Causal Analytics Toolkit (CAT).

Depending on whether data have been re-collected for the same colonies, causal models can also incorporate the temporal dimension. In this case, an array of methods, known as 'temporal causal models', can be used (Verdes, 2005; Arnold et al., 2007).

Some applications and examples related to bee health

All of these approaches refer to graphical models with directed relationships among the variables. In the case of bee health, these variables would be those identified in TOR3. Relationships among them would be modelled as directed causal paths that follow from well accepted theories, such as, for instance, the life history model (Fabian and Flatt, 2012). The mind maps in TOR2 suggest possible paths for a causal analysis of bee health.

An example of a causal model can be found in Le Conte et al. (2010) in which the cyclical causal relationship between *Varroa*, pathogens, beneficial microbes and bee health is clearly shown. Besides having a direct negative impact on bee health, *Varroa* increases bee infection with viruses, bacteria and so on, which then have a direct negative impact on health. Furthermore, these pathogens, in turn, increase susceptibility to *Varroa*, leading to a vicious circle. Another feature of this model is that beekeeper practices impact bee health only indirectly, through their effect on more proximal factors such as *Varroa*, acaricides, etc. Although the model shown is merely theoretical, its correctedness, in principle, can be empirically tested, provided adequate data.

Predictive multivariate analysis using Partial Least Squares (described under Approach 1, Appendix H) is a causal model. Appendix H includes an example in which there is a path between drivers and colony attributes and between colony attributes to colony outputs. The influence of each variable is modelled by linear combinations of variables (also seen as projections to a lower dimensional space).

Pro/cons of using causal models to analyse bee health

The advantage of a causal model is that it can, in principle, be 'asked questions' of all the types described in this paper, including descriptive, explanatory, predictive and prescriptive. For example, if the model-described relationships turn out to be strong empirically, a clear prescription would be to

endeavour to lower *Varroa* infection as much as possible. The disadvantage is that considerable amounts of domain knowledge are needed to correctly specify such a model.

Causal modelling is able to assess more than one health indicator at the time because its framework uses networks of variables and is designed to identify causal relationship between any number of variables. Causal modelling being a statistical model (as opposed to a population biology model), there is no specific requirement for a particular statistical unit: causal modelling, therefore, can use the colony as the unit of interest and assess bee health at different temporal and spatial scales. Assuming an accurate model specification, causal modelling can effectively detect early signs of deterioration in managed honeybee health. As a draw-back, a substantial amount of data is required to support data-driven causal modelling with many nodes and linkages, especially when linkages are also tested for.

Approach 4 – Predict bee health by process-based modelling

Description of the main characteristics and properties of process-based modelling

Process-based models (a.k.a. mechanistic models) express causal relations, non-linear dynamics and stochastic properties of systems (see e.g. Cuddington et al. 2013 for an introduction).

Complex process-based models may have many variables linked with non-linear equations or multidimensional stochastic processes. Random forager behaviour, random responses to external drivers and random fecundity and mortality of bee are features that result in a stochastic model, i.e. the model does not produce the same output all the time.

Individual Based Models (IBM) (or agent based models) is a class of stochastic models which seek to capture or predict emerging properties of a system by implementing behaviour at a higher level of detail (Railsback and Grimm, 2011; Grimm and Railsback, 2013). It can for example be to capture the development of a colony based on the decisions taken by individual foraging bees or individual growth and mortality of bees (e. g. as in the MUST-B project).

There are different and, to some extent, complementary ways to calibrate these types of models. Techniques for causal modelling apply here as well, because process-based models are also causal models. It is important to note that the structure of the process-based models (i.e. the variables and equations) is fixed, which is different from other models. The aim of the calibration is to inform the model parameters. Calibration usually starts with assigning parameter values based on an expert's knowledge, which in turn is informed by the peer-reviewed literature. Parameters are assigned numerical values with high precision (e.g. a specific number or a range) taking uncertainty into account (e.g. a probability distribution). Data associated with variables in process-based models are assimilated by adjusting the parameter values (or distributions) to optimise the model's ability to predict what has been observed. What is optimal here depends on the statistical objective function used. A Bayesian statistical objective for data assimilation is to update parameters with values that maximise posterior probability. Other objective functions are to assign model parameter numerical values that maximise a likelihood function, i.e. a probability mass or density of the parameter-given data, or minimise a loss function, for example, the sum of squares of predictive errors (Hastie et al., 2009).

The calibration of complex process-based models is complicated by the need to rely on multiple, not always associated, sources of data. Sometimes, calibration stops after the experts have assigned parameters. If so, it is important to test and possibly quantify the predictive accuracy of a model given the available data. Statistical calibration is the process where we make inference on model parameters based on data. Bayesian calibration (including inverse modelling) can be used to update parameter values considering both expert knowledge and the available data (Hartig et al., 2011, 2012; Jackson et al., 2015). Bayesian calibration treats parameters as uncertain and expresses this uncertainty by a probability (Gelman et al., 2014). For complex models, parameters are updated by sampling techniques. Algorithms, such as Markov Chain Monte Carlo sampling, allows us to sample from the high probability space for parameters instead of finding a complete analytical expression of a high-dimensional probability distribution (which can be extremely difficult when the numbers of parameters and equations are large). The use of Bayesian calibration techniques on complex models has increased during recent years due to the accessibility of fast algorithms to sample from the posterior. Examples of open source tools are BUGS (Bayesian inference Using Gibbs Sampling) and Stan (Hamiltonian Monte Carlo sampling). Approximate Bayesian computation offers a class of algorithms that may be as reliable and faster than full Bayesian methods.

However, the calibration of complex process-based models that require simulation to make predictions (e.g. when the model is a simulator defined by a computer code and does not have a closed analytical form) can be highly resource demanding. Examples of complex models are high-dimensional differential equations used to model global climate systems (Bhattacharya, 2007; Lee et al., 2011) or individual-based models used to model dynamics in a bee colony (e.g. .BEEHAVE, Becher et al., 2014). In that case, it is possible to replace a simulator by a metamodel (a.k.a. emulator or surrogate model) built for the purpose of calibration (Kennedy and O'Hagan, 2001; Oakley and Youngman, 2015). Regression models, response surfaces, neural networks, support vector machines and Gaussian processes are examples of models that have been used to build meta-models. Meta-models are widely used to approximate complex models and increase speed in computations and calibration (as e.g. in Andrianakis et al., 2015), but also in communication (Jalal et al., 2013; Lee et al., 2015).

Bayesian calibration can be carried out in a sequential way, which means that it is straightforward to continuously update parameters and validate the model when new data become available. Bayesian approaches for learning are useful for updating risk-assessment models based on monitoring data. Bayesian calibration of risk assessment models are also useful because uncertainty is quantified by probability, which can be propagated into the assessment models and make predictions with uncertainty and quantify impact of uncertainty on decision objectives (Cox, 2012; EFSA draft guidance on uncertainty[36]).

The representativeness of data for calibration determines the domain in which the model can be applied. For example, a model calibrated on normal conditions may fail to predict colony dynamics under extreme conditions. Data collection for calibration should therefore be proceeded by careful experimental design. Sensitivity analysis can be useful to identify which model parameters that has the largest impact in a model and for which reduction in uncertainty will lead to the highest improvement of the model.

Some applications and examples related to bee health

Modelling pesticide effect on bee health

The BEEHAVE model (Becher et al., 2014) is an IBM which consists of four modules: the colony module, the foraging module, the *Varroa* mite and virus module, and the landscape module. The colony model is a population dynamics model that simulates the development of cohorts of bees from eggs, larvae, in-hive bees and drones. This colony model is linked to environmental factors by the landscape module, resulting in seasonally dynamic storage, consumption, demand and collection of nectar and pollen. The foraging model is an agent-based model with forager squadrons as (super-) individuals.

The BEEHAVE model does not consider the effect of pesticides. The MUST–B WG recently published the specifications of a process-based model to assess risks to honeybee colonies from exposure to pesticides under different scenarios of combined stressors and factors affecting the health status of the colonies (EFSA, 2016b). Conceptually, the proposed model can be considered as a series of layers. The first layer represents a single honeybee colony in a complex landscape. The base model is composed of three interlinked modules: the foraging, colony and in-hive products modules; these are connected to the landscape which comprises two other modules: the RPU and the environmental driver modules.

Pollination service modelling

Potential pollination service has been analysed using statistical models, such as species distribution models that use habitat associations to map species abundance (Polce et al., 2013). Existing species distribution models for wild pollinators identify the distance to suitable habitats for nesting as the most important predictor of bee abundance. For honeybees, there is no need to model the position of the colony, because it is known. Furthermore, habitat association models do not explicitly take into account dispersal or foraging behaviour. Explicit foraging models are needed to quantify pollination service by managed honeybees.

Species abundance is not a measure of pollination service. Instead, the response is the intensity of visits to flowers in need of pollination and the extent to which this process is successful (see Section 3.4). Potential pollination service can be quantified by pollination service models that link spatially explicit land-use information with foraging behaviour to predict visitation rates at a resolution

[36] https://www.efsa.europa.eu/sites/default/files/consultation/150618.pdf (last accessed 8 July 2016).

higher than the crop field level (the invest model of Lonsdorf et al., 2009). Foraging models are used in individual-based models, such as BEEHAVE, but can be used as separate models predicting average foraging activities by a colony. Fine-level differences between landscapes and regions can be obtained by using models that consider optimal foraging behaviour (e.g. Olsson and Bolin, 2014; who deal with more complex foraging theory compared with Lonsdorf) or weather- and climate-related impact on foraging activity (e.g. Zulian et al., 2013 who take into account temperature and solar irradiance when modelling foraging activity).

Thus, honeybee visitation rates to pollinated flowers with high spatial resolution, and thereby their pollination service, can be assessed by process-based modelling using foraging theory in combination with land-use information within the RPU. As an add-on, a foraging model can be used to quantify the amount and quality of nectar and pollen resources brought into a colony. These model-based measures quantify the impact of land use in the RPU based on a mechanistic understanding and may complement the direct inclusion of land-use information in studies like Clermont et al. (2015), where the aim was to assess the impact of land use on colony attributes. In a similar way, foraging models can be used to provide more realistic assessments of exposure to potentially toxic chemicals, such as pesticides in crop fields within the RPU.

Pro/cons of process-based models to assess bee health

Process-based models are able to:

- base the assessment on more than one health indicator because they are developed *ad hoc* and can be tailored, in principle, to any data thought to be informative about the process. A limitation is that these models are computer demanding; use the colony as the unit of interest. However, considering the colony as the modelling unit may limit model performance because many processes relevant to the assessment of bee health take place at scale finer than that of the colony;
- assess bee health at different temporal and spatial scales, although the scale considered should be compatible with those underlying the bee biology;
- detect early signs of a deterioration in managed honeybee health.
- be built to be useful for a specific purpose, although it might not be useful for other purposes.