TECHNICAL REPORT

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A mechanistic model to assess risks to honeybee colonies from exposure to pesticides under different scenarios of combined stressors and factors

European Food Safety Authority

Abstract

A conceptual model for the risk assessment of pesticides in a single honeybee colony under different scenarios was developed. The model is composed of different modules. A base model representing the honeybee colony (the Colony, the in-Hive Products and the Foraging modules), placed within a complex landscape (the Resource Providing Unit and Environmental Drivers modules) and the inclusion of multiple factors and stressors on colony health (the Pesticides module, the Biological Agents module and the Beekeeping Management Practices module). The in-hive products comprise pollen and beebread, nectar and honey, water, jelly and wax. The foraging module is linked to the colony (food stores), resource-providing unit (availability of resources in terms of protein and sugar amounts) and environmental drivers. It is based on forager decisions (to fly or not and to collect nectar, pollen or water). The Pesticides module includes exposure (both outside and in-hive) to pesticides and effects in bees (queen, larvae and pupae of drones and workers, nurses and other in-hive bees, foragers and winter bees). Scenarios comprise different landscapes, weather and climatic conditions, some biological agents and beekeeping management practices having an influence on the dynamics of the colony and in-hive products. The spatial scale is 3 km around the hive and the spatial resolution is 1 m^2. The temporal scale is 1 year corresponding to one colony annual cycle and the temporal resolution ranges from hours (e.g. inflow of pollen/nectar/pesticides) to days (in-hive processes). Recommendations were made for the development of the conceptual model presented in this report into a mechanistic model to assess the risk of pesticides on honeybee colony health in complex landscapes and in the presence of multiple stressors. Finally, new opportunities for further model implementations were highlighted.

Key words: honeybee colony, risk assessment, modelling, landscape, multiple stressors, pesticides, biological agents

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Summary

Continuing the horizontal work initiated by the internal and multidisciplinary task force (i.e. the Bee TF) in the area of bee health, the Scientific Committee and Emerging Risks (SCER) established a large project MUST-B (EU efforts towards the development of a holistic approach for the risk assessment on MUltiple STressors in Bees) and a working group (MUST-B WG) of the Scientific Committee. This is in line with the role of the European Food Safety Authority (EFSA) to develop integrated risk-assessment approaches and to move towards an integrated and holistic approach for the risk assessment on multiple stressors in bees.

The MUST-B project comprises three *ad hoc* EFSA working groups of experts, MUST B, BEEHAVE and HEALTHY-B. The BEEHAVE working group of the EFSA Panel on Plant Protection Products and their Residues assessed the suitability of the BEEHAVE model for its potential use in a regulatory context and for the risk assessment of multiple stressors in honeybees at the landscape level. The MUST-B working group further considered results and recommendations from the EFSA Panel on Plant Protection Products and their Residues. The HEALTHY-B working group of the EFSA Panel on Animal Health and Welfare elaborated a toolbox (indicators, variables and methods/tools) to assess the health status of a honeybee colony in large field surveys.

MUST-B intends to develop a framework incorporating modelling, experimental and field-monitoring approaches to extrapolate risks from individual to colony levels and assess the relative contribution to colony weakening and losses of multiple stressors from both the in-hive environment and the surrounding landscape.

Modelling is at the core of the MUST-B framework. The model should be developed as a quantitative tool for regulatory risk-assessment (RA) purposes and as a predictive and explanatory tool to better understand the (relative) risks and impacts of multiple stressors on honeybee colonies, including the overall complexity of interactions.

According to EFSA opinion on good modelling practice, the MUST-B working group developed a conceptual model (qualitative description of the system to be modelled: insights into the environmental and biological processes and their interactions and interdependencies) which provides the basis for the development of a mechanistic model. The objective of the mechanistic l model is to assess the risks from pesticide exposure on a single honeybee colony. The model outputs should include colony size and mortality of the various honeybee life stages (queen, drones and workers such as foragers, in-hive bees, larvae and pupae) and egg-laying rates.

The overall conceptual model (Section 5) is a series of layers. A base model representing the honeybee colony [Foraging module (Section 6) and the Colony and in-Hive Products modules (Section 7)], placed within a complex landscape [the Resource Providing Unit and Environmental Drivers modules (Section 8)] and the inclusion of multiple factors and stressors on colony health [the Pesticides module (Section 9), the Biological Agents module (Section 10) and the Beekeeping Management Practices module (Section 11)].

The foraging module describes the decision of a forager bee to leave or rest and to forage and collect pollen, nectar or water (Figure 6). The honey and pollen stores and availability of food sources in the landscape (distance to hive and quantity of nectar and pollen provided) influence the decisions. Colony needs, environmental drivers, and the characteristics of the Resource Providing Unit determine the foraging activities. Given the lack of data on all possible effects on foraging behaviour, only impacts on homing and age of first foraging are taken into account in this module. The temporal scale is 1 year corresponding to one colony annual cycle and the time step goes from hourly (food brought back to the colony) to days (for summed forager mortality).

The Colony module describes demographic (egg-laying, mortality, development of brood, comprising larvae and pupae of workers and drones, and adults), behavioural (nursing, non-nursing, foraging and thermoregulating in winter) and physiological (energy requirements and susceptibility) traits. Key variables include colony size and demographic structure.

The Foraging module and the Colony and in-Hive Products modules are the core modules of the model. The in-hive products are categorised according to how bees handle and/or consume these products (Table 2). They comprise fresh pollen (stored pollen < 1 day old), beebread (stored pollen >

1 day old), fresh nectar (nectar < 1 day old, i.e. either transported by foragers or stored in cells), freshly stored nectar (nectar stored in cells between 1 and 3 days old), honey (nectar stored in cells > 3 days old) and jelly. Finally, a conceptual model is provided to determine how the in-hive products are processed into food by bees (Figure 10).

For the higher tier risk assessment of pesticides, the influence of the landscape, weather and climate on the honeybee colony (effect on foraging decisions and colony dynamics) needs to be considered. The Resource Providing Unit and Environmental Drivers modules describe the landscape structure and dynamics. The weather and climatic conditions must be described on an hourly basis and at different spatial scales: the cell (1 m^2 resolution), the 'patch' (series of cells in an unique habitat) and the resource providing unit (an area with a radius of 3 km around the colony comprises a series of patches). In particular, hourly availability of pollen (i.e. weight/weight % protein), nectar (i.e. weight/weight % sugar) and water (absence or presence) and related concentration of pesticides in crops in the cells should be determined. The landscape structure must comprise data on land use (e.g. vegetation type, non-vegetated areas and soil type), patch characteristics (e.g. size, shape and boundaries), and colony location. The landscape dynamics must comprise data on weather (temperature, daylight, relative humidity, total precipitation, wind direction and speed), climate (thermal sums, average temperature, average precipitation and snow cover) and pollen, nectar and water availability and pesticides concentrations in these matrices (based on information collected from plant phenology, farm management and plant pest control actions) at the appropriate time resolution.

The conceptual module for pesticides is based on pesticides exposures (in the landscape: exposure of foragers and in-hive: exposure of all bees) and effects resulting from these exposures for all bee life stages to the exception of eggs. Due to high temporal variability of pesticides concentrations in fields (and nectar, pollen and water) after spray application, 1 h-time step is recommended for contact exposure and concentrations in nectar, pollen and water (i.e. surface, puddle and guttation). Pesticides consumption effects need to consider three conceptual processes: the processing of food by bees, pesticide processes in food, and consumption of food (see Figures 12 and 14, and Table 4). The Ecotoxicologically Relevant Exposure Quantity (EREQ) describes the link between consumption and effects, which is the interface between different exposure and effects assessments (Table 5). For each bee life stage, dose–response relationships are required (where EREQ is the dose and percentage or probability of mortality is the response; Figure 17). For non-toxic substances, No Observed Effect Level (NOEL) are required as input for the model. While it is assumed that effects are the same in all adult bee stages, acute and chronic effects in winter bees *versus* summer bees and effects in larvae *via* both oral and contact exposures need to be taken into account in the model. However, further investigation is required on how to account for sublethal effects (e.g. reproductive performance of queens, ability of nurses to feed larvae and ability of foragers to perform appropriate homing behaviour).

The influence of *Varroa destructor* and its two associated viruses (i.e. the Deformed Wing Virus, DWV and the Acute Bee Paralysis Virus, ABPV) and *Nosema* spp. are documented for inclusion in the Biological Agents module. Based on expert knowledge, the working group identified the different colony traits affected by each of these biological agents. Additional information and references were provided on future perspectives for the modelling of biological agents and on general considerations regarding the life history of *Varroa* and *Nosema* spp., but since they were not of direct use for the model presented in this report, this information was moved to the appendix section (Appendices A and B).

The Beekeeping Management Practices module is based on a first listing and scoring by the HEALTHY-B working group. Six beekeeping management practices were selected to be included in the model, namely 'change in number of workers', 'chemical control', 'replacement of combs with brood', 'replacement of combs with feed sources', 'supplementary feeding' and 'beekeeper category and experience'. For each of these beekeeping management practices, a definition was provided and their effects on the colony and in-hive products module were further documented, as for the Biological Agents module, with diagrams (see Sections 11.2.1 to 11.2.6) and scientific literature (see Appendix C). For 'beekeeper category and experience', it was assumed that for risk assessment and testing purposes, only optimal 'good beekeeping practices' would be followed.

The Resource Providing unit and Environmental drivers, the Biological agents and the Beekeeping Management Practices modules impact on the honeybee colony population should be described by

means of a scenario-based modelling. Further model developments could consider processes-based dynamical description of what has been considered only as a scenario (e.g. see Appendix A for population dynamics of the biological agents and their interaction with the honeybee colony population).

Finally, recommendations for future development are made. The working group listed some areas for further development in each of the modules and described how the constraints identified by EFSA Panel on Plant Protection Products and their Residues on the BEEHAVE model were addressed by MUST-B. The working group made substantial progress towards the development of a model that is broader in scope and has the capacity to expand in the future. Recommendations are made for the development of a mechanistic model to be used to assess the risk of pesticides on honeybee colony health in complex landscapes and in the presence of multiple stressors. To assess the performance of the model, a precise field data collection will be conducted.

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1. Introduction

1.1. Background and Terms of Reference as provided by EFSA

1.1.1. Background

The European Food Safety Authority (EFSA) has the mandate to improve food safety in Europe and to ensure a high level of protection of human and animal health, including bee health, and the environment, including ecosystem services such as pollination of a wide range of crops and wild plants, which is largely provided by bees.

The way that stressors (biological, chemical and environmental) affect bees and contribute to the current observed trends of population declines is not well understood, neither are the underlying mechanisms, which remain complex given the potential number of combinations and interactions among stressors.

In 2008, EFSA conducted a survey on existing bee surveillance systems in the European Union (EU) (EFSA, 2008). Following its recommendations, the European Commission (EC) established an EU Reference Laboratory (EURL) for honeybee health (Commission Regulation (EU) No 87/2011[1]) and funded an EU-wide monitoring programme on honeybee mortalities and diseases in Europe (EPILOBEE). The results of this programme showed a geographic north-south trend in mortality (Chauzat et al., 2014), but given the large dataset, high number of variables not yet fully analysed, and the absence of data on the monitoring of other bee stressors (i.e. chemical and environmental factors), these results remain preliminary.

At EFSA, the multifactorial aspect of bee losses and colony weakening puts this issue under the competence of the Scientific Committee, which addresses multisectorial issues (Article 28 of EFSA's Founding Regulation (EC) No 178/2002[2]). It is the role of the Scientific Committee and Emerging Risks (SCER) unit to develop integrated risk assessment approaches (EFSA, 2015).

In 2012, in line with its mandate, the SCER unit initiated horizontal work in the area of bee health through the establishment of an internal and multidisciplinary task force (i.e. the Bee TF) and through the organisation of a scientific colloquium (EFSA, 2013a). The Bee TF produced an inventory of EFSA's work in the area of bee health (EFSA, 2012) and consulted a wide range of stakeholders (i.e. the European Commission and Member States) to identify knowledge gaps in research and to make recommendations to move towards an integrated and holistic approach for the risk assessment on multiple stressors in bees (EFSA, 2014). Some specific recommendations were made for future work at EU level, which is further described below.

1.1.2. Terms of Reference

This project comprises several interlinked activities (see overview Figure 1) to be either continued (e.g. use of in-house expertise through the Bee TF and development of outsourcing projects and networking activities) or developed (e.g. scientific support from the Scientific Committee and external experts, collaborations with EURL on honeybee health and outsourcing the collection of scientific evidence for risk assessment/monitoring on multiple stressors in bees).

[1] Regulation (EU) No. 87/2011 415/2013 as from 6 May 2013. OJ L 29, 3.2.2011, p. 1–4.
[2] Regulation (EU) No. 178/2002 as from 28 January 2002. OJ L31/17, 1.2.2002, p. 1–24.

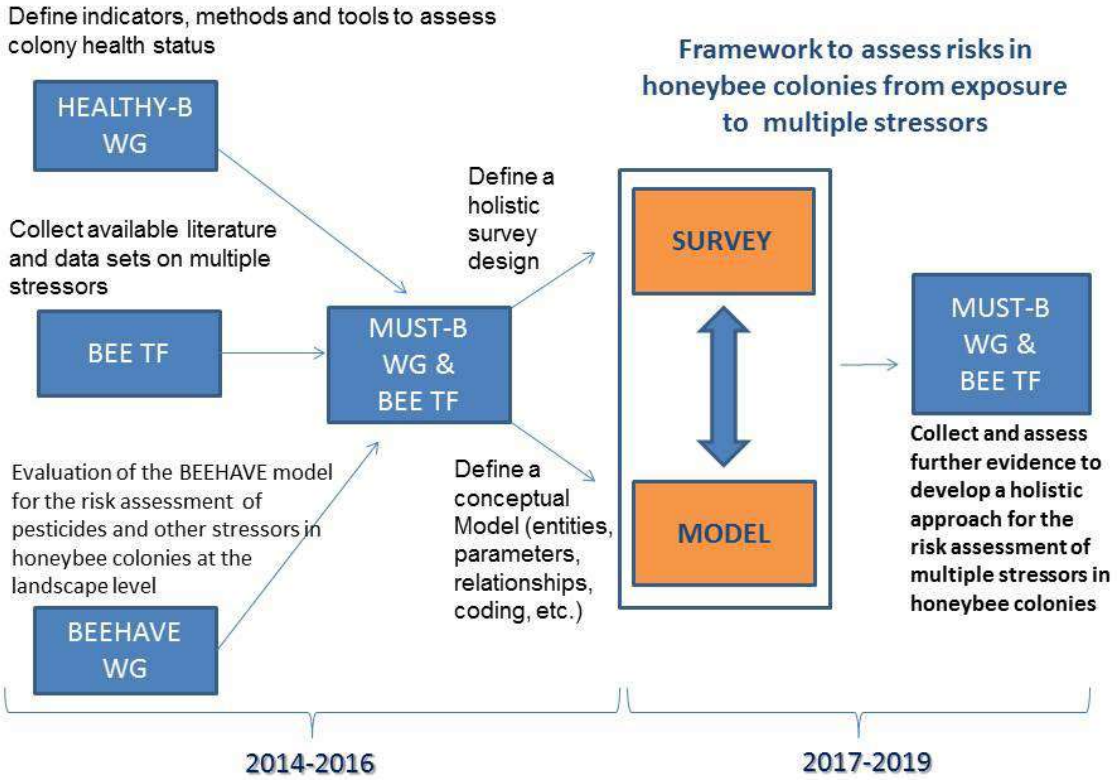

Figure 1: MUST-B project 2014–2019

The final goal of this project is to bring together all available expertise and knowledge in the area of bee health and risk assessment by further developing the multidisciplinary approach initiated at EFSA by the bee task force. This will be moved forward by bringing together evidence and stakeholders for a more cohesive and collaborative approach towards the development of a holistic approach to the risk assessment on multiple stressors in bees.

The Working Group (MUST-B WG) of the Scientific Committee (SC) and the EFSA Bee Task Force (TF) have been given the following tasks:

- To develop a holistic approach for the risk assessment on multiple stressors in honeybee colonies; this will be formalised through a Scientific Opinion of the Scientific Committee. Technical reports will be produced through the analysis of specific data sets as they become available by outsourcing and other activities.
- To produce regular updates on EFSA's activities in the area of bee health.

The Bee TF is a multidisciplinary group of EFSA staff that supports all MUST-B activities and regularly reports on MUST-B progress and other relevant on-going activities, dealing with bee-health issues *via* a dedicated microsite on the new EFSA portal.

1.2. Interpretation of the Terms of Reference

1.2.1. Background

Assessing risks to populations from multiple stressors is a great challenge because of the considerable uncertainty about how such assessments should be conducted (Munns, 2006). In order to tackle such a scientific topic, the United States Environmental Protection Agency (US-EPA) suggests an approach that requires the contribution of three scientific disciplines (ecotoxicology, population biology and landscape ecology) and research on predictive modelling to extrapolate from individual to populations and communities, between species, and at various spatial and temporal scales (Figure 2). The US-EPA approach was developed for a broader context (i.e. wildlife risk assessment research) than the one

developed by MUST-B (honeybee colony risk assessment). However, it does provide insights about conducting the environmental risk assessment of multiple stressors on honeybee colonies at the landscape level. There is evidence that elements from the fields of ecotoxicology, population biology and landscape ecology all have an influence on, and are therefore relevant to understanding, the relative importance of single and combined stressors.

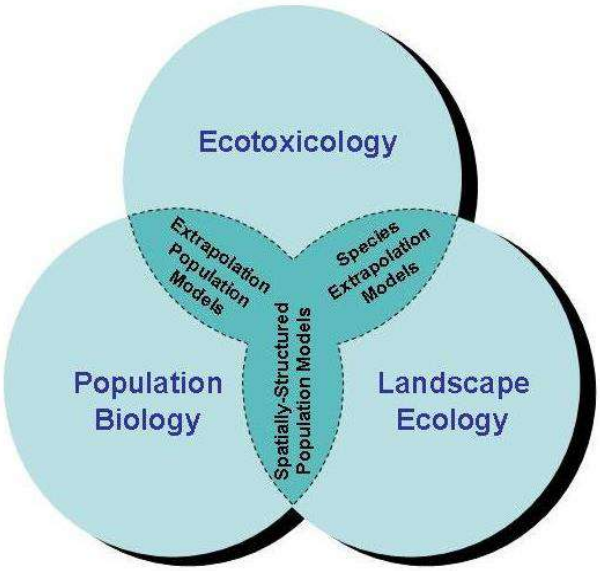

Figure 2: US-EPA research strategy to assess risks to populations from multiple stressors (from Munns, 2006)

1.2.2. The work of the MUST-B working group

The MUST-B WG is developing a framework incorporating modelling, experimental and field-monitoring approaches. These complementary approaches are being combined to extrapolate risks from individual to colony levels, to assess the complexity of co-exposures from multiple stressors coming from both the hive environment and the landscape, and to determine their relative contribution to colony losses and weakening. The work is being conducted with input from the disciplines of ecotoxicology, population biology and landscape ecology.

Modelling is at the core of the proposed framework. As highlighted previously, mechanistic models represent a powerful tool to simplify and assess the complexity of multiple stressors on honeybee colony health (EFSA, 2013a). To be useful, however, such models need to be calibrated with high quality data collected *via* experimental studies and further validated in wider environmental contexts *via* monitoring of a wider range of stressors and factors known to have an impact on colony dynamics.

The current technical report outlines specifications for model development, drawing extensively on expert knowledge and a detailed understanding of current published information. A second technical report is currently being finalised outlining specifications for field data collection, contributing to post-model development validation.

It is envisaged that the model will be used as an exploratory tool for regulatory risk assessment (RA) purposes and also to better understand the (relative) risks and impacts of multiple stressors on honeybee colonies, including the overall complexity of interactions. Such a model would not address all multiple stressors, but rather would be a mechanistic model designed to assess the risks from pesticide exposure on a single honeybee colony in a complex landscape, after considering different scenarios of biological agents and beekeeping practices (Figure 3). This technical report will support work towards the development of the computer model, as outlined in the bottom orange box in Figure 3.

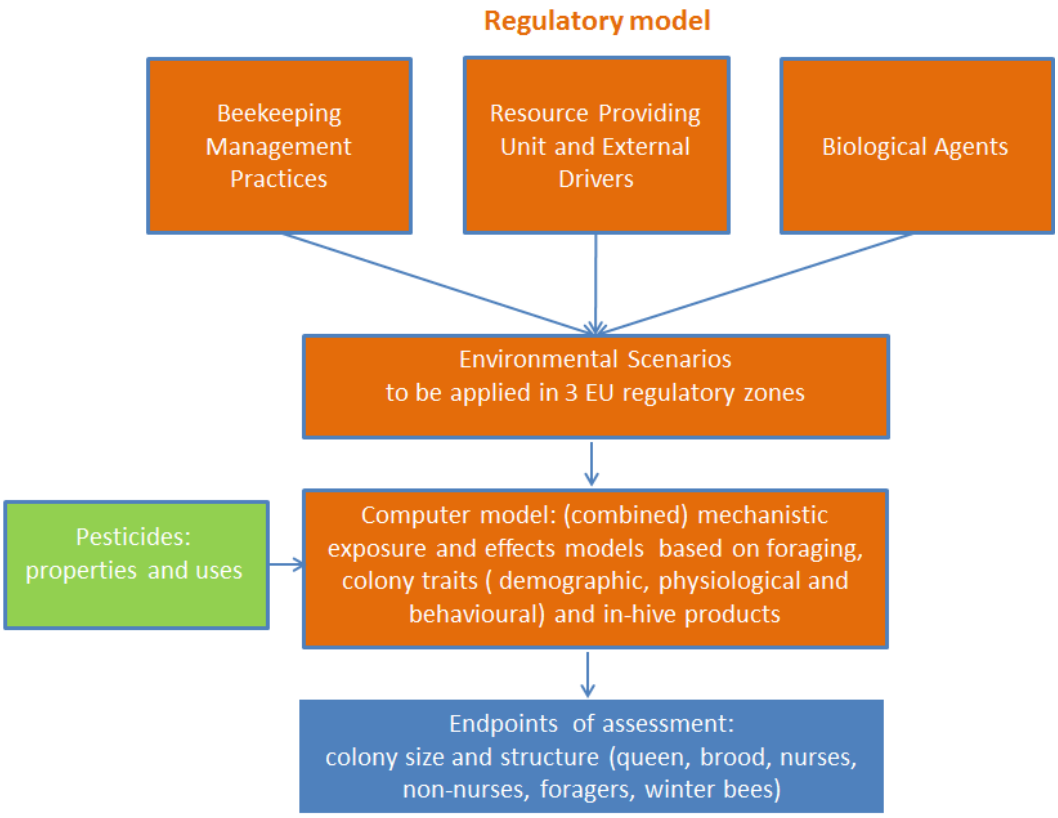

Figure 3: Schematic representation of the regulatory model for the risk assessment of PPP on honeybee colonies at the landscape level (adapted from EFSA PPR Panel, 2014). 'Brood' includes larvae and pupae of drones and workers

1.2.3. Related activities

As shown in Figure 1, the MUST-B WG is supported by two WGs (BEEHAVE and HEALTHY-B) and the Bee TF, which generate data, results and/or recommendations relevant for the work of the MUST-B WG:

- The BEEHAVE WG of the EFSA PPR Panel carried out a stepwise evaluation of the BEEHAVE simulation model (Becher et al., 2014) according to the EFSA opinion on good modelling practice (EFSA PPR Panel, 2014) to assess its suitability for use in a regulatory context and for the risk assessment of multiple stressors in honeybee colonies at the landscape level (EFSA PPR Panel, 2015). The BEEHAVE model simulates the hive population dynamics by considering (i) environmental factors, such as weather conditions, distance to patches[3] and food availability (pollen and nectar), that may influence foraging ability and biological agents (the *Varroa* mite and two associated viruses, the deformed wing virus – DWV – and the acute bee paralysis virus – ABPV– and (ii) other population-dynamics parameters that may impact the colony development. The way the MUST-B WG addressed the recommendations made by the EFSA PPR Panel for the implementation of the model are described in section 5.
- The HEALTHY-B WG of the EFSA AHAW Panel defined indicators, variables and methods/tools to assess the health status of a honeybee colony in large field surveys (EFSA AHAW Panel, in preparation).
- The Bee Task Force brought further sources of information in the form of literature reviews and datasets (Thompson, 2012; ANSES, 2015; Jacques et al., 2016) to be further processed by the MUST-B WG.

[3]A relatively homogeneous area that differs from its surroundings (Forman, 1995) – see Glossary for a more detailed definition

In addition to the BEEHAVE model, the Dynamic Energy Budget (DEB) and Animal, Landscape and Man Simulation System (ALMASS) models were presented to the MUST-B WG by experts in the field of modelling applied to ecotoxicology, population biology and landscape ecology. The usefulness of such approaches was further discussed within the WG.

2. The modelling cycle

The MUST-B WG agreed that model development should follow the EFSA opinion on good modelling practice (GMP), which describes the steps involved in developing a model for use in environmental risk assessment (EFSA PPR Panel, 2014).

In line with the modelling cycle proposed by the EFSA Plant Protection Residues (PPR) Panel (Figure 4) the MUST-B WG has concentrated on the first three steps of the modelling cycle, which go from 'reality problem' to 'problem definition' to 'conceptual model'.

The conceptual model provides a qualitative description of the system to be modelled (insights into the environmental and biological processes and their interactions and interdependencies).

The different modules can be considered components of the overall model. Each was elaborated with expert knowledge. During this exercise, it is important to note that not all the literature cited in this report was critically assessed (and it was not following the principles of a systematic review). Rather, the references were used as examples to illustrate the processes involved within each module.

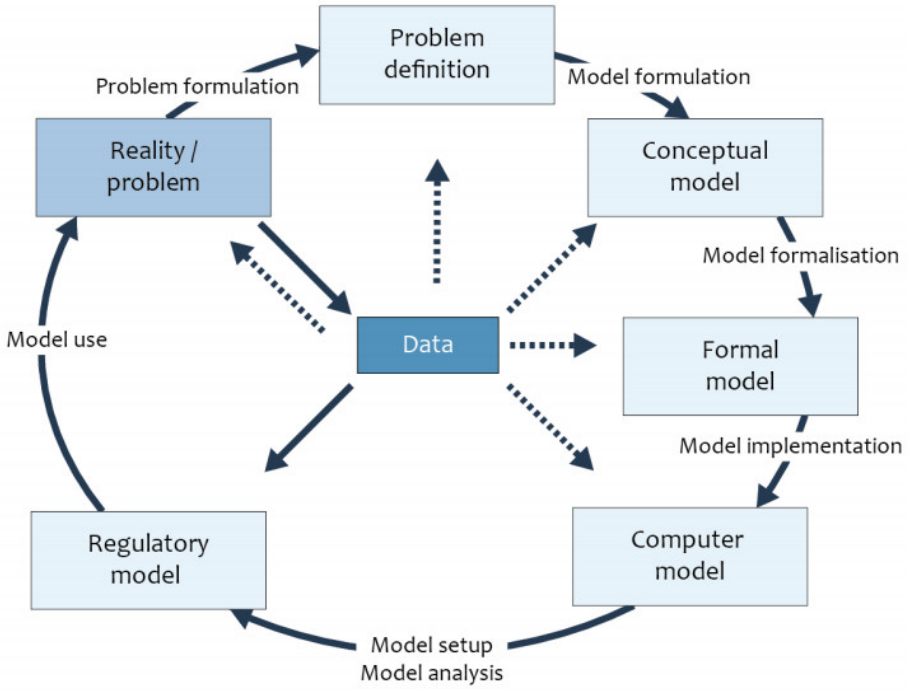

Figure 4: Modelling cycle from EFSA PPR Panel (2014)

3. Reality/problem

Honeybee-colony weakening and losses have been reported in the EU and worldwide (Neumann and Carreck, 2010; Potts et al., 2010; vanEngelsdorp et al., 2012a,b; van der Zee et al., 2012; Greenpeace, 2013; Cepero et al., 2014).

The biological agents and pesticides are the most frequently cited stressors to explain colony losses and weakening and there are potentially interactions among and between them (Oldroyd, 2007; VanEngelsdorp and Meixner, 2010; VanEngelsdorp et al., 2010; Neumann and Carreck, 2010; Potts et al., 2010; Nazzi and Pennacchio, 2014; Goulson et al., 2015; Simon-Delso et al., 2015). The existence of such interactions is supported by studies showing the multiplicity and high prevalence of multiple biological agents (Cox-Foster et al., 2007; Hedtke et al., 2011; Dainat et al., 2012a) and pesticides

(Chauzat et al., 2009; Mullin et al., 2010; Lambert et al., 2013; Paradis et al., 2013; Bonmatin et al., 2015; Pisa et al., 2015) in honeybee colonies.

Studies that examined the way these factors interact showed possible synergistic interactions, which are defined as a combination of stressors that results in a greater effect than expected from cumulative independent exposures (Holmstrup et al., 2010). Such interactions have been identified between chemicals (Colin and Belzunces; 1992; Johnson et al., 2009; Zhu et al., 2014), between biological agents (Dainat et al., 2012b; Nazzi et al., 2012; Toplak et al., 2013) and between biological agents and chemicals (Alaux et al., 2010; Vidau et al., 2011, Aufauvre et al., 2012; Boncristiani et al., 2012; Gregorc et al., 2012; Pettis et al., 2012, 2013; Locke et al., 2012; Wu et al., 2012; Di Prisco et al., 2013; Aufauvre et al., 2014; Doublet et al., 2015a; Retschnig et al., 2014). It is still unknown, however, what is the relative importance of these factors to explain honeybee-colony losses and weakening.

When assessing the risk of pesticides to honeybee colonies, a tiered approach is followed going from the most conservative (on individual bees and under laboratory conditions) to the most realistic (on colonies and under (semi)field conditions. Current field-testing would, however, need major improvements to detect significant effects (in bee mortality and disruption of the colony activity) with high statistical power and validated tools and methods (EFSA PPR Panel, 2012). Current tests do not reflect well the exposures of real colonies, which vary in time and space within a complex landscape. This is, in particular, because semi-field tests are too short in duration and tests in the field have plot sizes that are too small. Indeed, it is difficult and extremely resource demanding to test such complex exposure scenarios in the field. Another challenge, when doing risk assessments in honeybee colonies, is the extrapolation of effects observed on individual bees from the different categories of bees[4] in the hive (i.e. larvae, in-hive bees including nurses, foragers and the queen) to effects at the colony level. This is particularly a challenge for sublethal effects such as hypopharyngeal gland (HPG) development and homing behaviour.

With the above context in mind, the development of a mechanistic model reflecting this complexity was felt to be potentially a useful tool for the risk assessment of honeybee colonies exposed to multiple stressors that vary in time and space, at the landscape level (EFSA, 2013a).

4. Problem definition

Protection goals (PGs) determine what to protect, where and over what time period. In 2012, the EFSA PPR Panel defined protection goals for honeybee colonies, based on the ecosystem-services approach and using the approach outlined by the EFSA PPR Panel (EFSA PPR Panel, 2010). Pollination, hive products and biodiversity (specifically addressed under genetic resources and cultural services) were identified as relevant ecosystem services to protect. The EFSA Animal Health and Welfare (AHAW) Panel also identified these services as relevant to ensure the health of a managed honeybee colony. More specifically, according to the AHAW Panel, a colony was felt to be healthy: (i) when it has an adequate size, structure and behaviour in relation to the annual life cycle of the colony and the geographical location; (ii) when it has a normal production of bee products such as honey; and (iii) when it provides pollination services (EFSA, 2016; EFSA AHAW Panel, in preparation).

For each of the above general protection goals, the PPR Panel defined more specific protection goals in four dimensions (ecological entity, attribute, magnitude of effect and temporal scale) (EFSA PPR Panel, 2012). The PPR Panel defined the 'honeybee colony' and 'foragers' as the relevant ecological entities for pollination, hive products and biodiversity.

The AHAW and PPR Panels defined several key attributes (Table 1). Most of these attributes overlap, albeit with differences in wording.

[4]Different categories of bees may include: castes (workers, queen, drones), developmental stages (eggs, larvae, pupae, adults) and temporal tasks of the adult workers (nurses, foragers).

Table 1: Corresponding attributes to be protected in honeybee colonies according to the AHAW and PPR Panels

AHAW Panel attributes and correspondence with PPR Panel attributes
Queen performance (AHAW) => Reproduction (PPR)
Behaviour and physiology (AHAW) => Bee behaviour (PPR)
Demography (AHAW) => Survival and development of colonies; Effects on larvae; Abundance/biomass (PPR)
Hive products (AHAW) => not relevant/considered (PPR)
Biological agents (AHAW) => not relevant/considered (PPR)

In addition, besides exposure to plant-protection products (and other environmental pollutants), the AHAW Panel listed other factors that are known to have an impact on the health of honeybee colonies (i.e. environmental drivers such as weather and climate; resource-providing unit including resources availability, land use and cropping practice; beekeeping management practices) and which are part of the environmental scenario.

For each attribute, the AHAW Panel defined indicators that are measurable response variables and further considered for inclusion in the model by the SC Panel. For each of these variables, specific methods/tools will be identified and reported in a data model to be used for model calibration and validation with field data.

As for the magnitude of effects and temporal scale, the agreed protection goal (PG) by risk managers for honeybees exposed to plant-protection products was that effects on colony size above 7% (negligible) were not allowed (EFSA, 2013). In addition, it was also recommended that average daily mortality of foragers is not increased by a factor of more than 1.5 in 6 days, a factor of 2 in 3 days or a factor of 3 in 2 days. The overall level of protection also considers exposure-assessment goals. In the case of plant-protection products, the exposure assessment goal was set at the 90th percentile of colonies placed at the edge of treated agricultural fields. To meet this PG, the exposure should not exceed a level that could lead to effects greater than 7% in 90% of the colonies at the edge of treated agricultural fields. Whether effects are likely to be observed in the remaining 10% of the colonies at the edge of the field depends on the margin of safety identified in the risk assessment for the specific compounds. For example, if a compound is of low toxicity to bees and the risk assessment shows a large margin of safety, then there will be no effects even if the exposure exceeds the 90th percentile, but if the effect assessment indicates a narrow margin of safety then it is likely that effects are observed when the exposure exceeds the 90th percentile.

These protection goals need to be considered during model development. Key model outputs should include colony size (to allow assessment of changes in colony size caused by pesticides and interactions with other factors and stressors all through the year, i.e. in summer[5] and winter bees), the mortality of queen, drones and workers such as foragers, in-hive bees (nurses and non-nurses involved in various in-hive activities other than brood care), immatures (larvae and pupae) and egg-laying rates.

[5] Summer bees include all bees during the active season of honeybee colonies (i.e. from spring to autumn)

5. Overall conceptual model

In the earlier statement made on the use of the BEEHAVE model to assess risks of pesticides in honeybee colonies (EFSA PPR Panel, 2015), a number of methodological constraints and biological issues were identified, precluding the use of that model at that time for regulatory risk assessment. These constraints were used as a starting point in this work, highlighting critical areas where progress was needed. Most of these concerns have been addressed by the MUST-B WG, as follows:

- Absence of a pesticides exposure in BEEHAVE: the MUST-B working group elaborated a conceptual module for pesticides exposure to be developed in the model.
- Lack of realism of the potential effects of *Varroa* on the colony and absence of *Nosema* in BEEHAVE: the MUST-B working group elaborated a conceptual module for the inclusion of effects from different biological agents. In particular, the working group provided extensive information based on literature, though not systematic, on the various effects of *Varroa*, the DWV, ABPV and *Nosema* spp. on the colony.
- Absence of potential impacts from trade of bees and/or colonies in BEEHAVE: the working group included the beekeeping management practice 'change in number of bees' in the model which, in some instances, can be linked to trade. Another practice linked to trade is 'introduction of a queen bee'. While the working group did not consider it relevant for the model (when the user is following the good beekeeping practices and when it is used for the risk assessment of pesticides), the working group provided information on this practice in case it needs to be included in future development of the model.
- Lack of realistic landscapes in BEEHAVE: the RPU-ED modules outline the requirement of detailed spatial and temporal field data from defined study sites in Europe, and the contribution of these data to pollen, nectar and water availability, pesticide contamination and foraging behaviour.

Some recommendations could not be addressed by the working group because of lack of data, in particular for the following items the working group did not provide solutions:

- Effects of genetics of bees on colony sensitivity to pesticides and resistance to diseases, in egg-laying rates, foraging distances and pollen preference. The MUST-B working group concluded that the critical point in studies investigating the effect of the colony genetic origin is the lack of evidence. Although there is some evidence of genetic effects on the above traits, the MUST-B working group concluded that the number of studies is not sufficient to be able to report general trends of measurable differences between subspecies or strains that could be reliably used within a model. Furthermore, several studies have shown that genotype-environment interactions play a strong role in colony performance and fitness, thus the genetic origin should always be considered together with the location of the colony.
- Effects from movement of colonies and/or bees. The MUST-B working group developed a model for the risk assessment of pesticides on a single colony. Effects from movement of bees refer to disease spread and/or transmission between colonies and/or apiaries. Since the model is developed for a different purpose, those effects were not found relevant.

However, the model structure is ready to be adapted to include solutions for those issues whether and when data will be available.

5.1. Model structure

The design of the model comprises the following high-level requirements:

- The model is a mechanistic exposure/effect model based on the dynamics of the colony and the in-hive products and their interactions with the environment, which include the resources for the bees, the pesticides, the biological stressors and the beekeeping practices.
- The model includes a number of inter-linked modules, to be developed as a series of layers:
    - The base model comprises the core modules: the Foraging module (see Section 6), the Colony and the in-Hive Products modules (see Section 7). The Foraging and Colony modules are dynamic and based on energy budget at the individual level.

Biological processes are described in terms of demographic traits (development, fecundity, and mortality) and physiological and behavioural traits; the in-Hive Products module describes the process of inflow, maturation, storage and outflow of in-hive products.

  - The Resource Providing Unit and Environmental Drivers (RPU-ED) modules (Section 8).
  - The Pesticides module is based on dose–effect relationships showing how the different types of individuals will be affected by the pesticides concentrations in bee products (Section 9).
  - The Biological Agents module, comprising *Varroa* with the two associated viruses (DWV, ABPV) and *Nosema* spp. (Section 10 and Appendix A for population dynamics approach and Appendix B for reviews on *Varroa destructor* and *Nosema* spp.).
  - The Beekeeping Management Practices (BMP) module (see Section 11 and Appendix C for reviews).

- Some of the modules (C-HP and Foraging) will be based on a fully mechanistic description of the processes. Other modules (RPU-ED, Biological Agents and BMP) will be represented as a series of potentially realistic 'scenarios'.
- The model will be run at a series of defined sites, using realistic data for the RPU and ED from each of these sites.

It is proposed that the model is developed as a series of layers, initially a base model representing a single honeybee colony, then the placement of the colony within a complex landscape, and finally the inclusion of one or multiple factors and stressors on colony health (Figure 5).

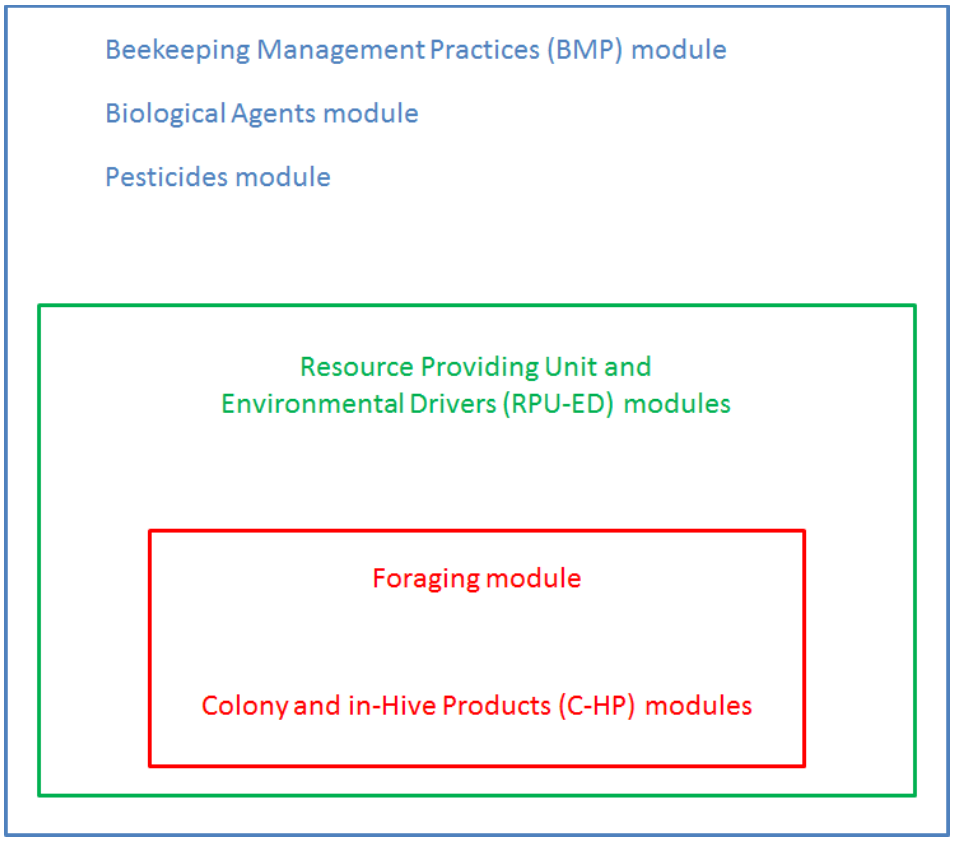

Figure 5: Overview of the layered approach for the development of the model

5.2. Scenario-based approach for the modelling of the RPU-ED, biological agents and BMP

The model here proposed is designed to assess the risks from pesticide exposure on a single honeybee colony in a complex environment, considering also the contribution of biological agents and beekeeping practices. Understanding process-based models results can be limited by model complexity, expressed in terms of number of interacting processes and functions to be considered. To account for the need of realism in system representation and understandability of simulation results the modelling strategy here adopted restricts the mechanistic exposure/effect model to the dynamics of the honeybee Colony, the Foraging and the in-Hive Products modules.

For some of the modules (RPU-ED, Biological Agents and BMP) a fully mechanistic description of the processes is not given, instead they are represented as a series of potentially realistic 'scenarios'. Here, a scenario is intended as any plausible combination of state variables and their dynamics describing a process, which is designed to evaluate the implications of alternative possible situations to be assessed. In the context of the risk assessment of plant protection products, environmental scenarios are required and defined by EFSA PPR Panel (2014) as a combination of abiotic, biotic and agronomic parameters, or as a conceptual and quantitative description of the environmental system relevant to the risk assessment, including the habitat (at relevant spatial and temporal scales) and the driving environmental variables including external stressors.

Scenarios are designed to reasonably characterise the range of variability in the environment, the biological agents and the BPM, and the influence of such variability on honeybee colony population dynamics, behaviour and physiology under exposure from pesticides.

The influence of RPU-ED, biological agents and BMP on the honeybee colony population is described by means of a scenario-based modelling approach in which:

(i) Scenario variables are external forcing variables (i.e., driving variables) used to describe the time-dependent effect of RPU-ED, biological agents and BMP on pre-defined target processes. A forcing function is a function that appears in an equation and is only a function of time, not of any of the other variables. An example of a forcing variable is the population abundance or the prevalence of *Varroa* mites and the mortality of larvae and pupae is the target process;

(ii) The scenarios related to RPU-ED, biological agents and BMP and their dynamics in space and time are described in terms of the spatial and temporal pattern of the forcing variables;

(iii) The effect of a forcing variable is to drive the change of a target process toward a different state which is dependent on the value of the forcing variable.

A number of scenarios have to be created which reasonably characterise the range of forcing variables for the environmental mechanisms being studied, the range of biological agents to be considered and the change in their prevalence during the year, as well as the set of measures implemented by beekeepers.

These scenarios are created to provide a representative combination of information on land use, crop/wild plants distribution and productivity, climate, meteorology, micro- macro-parasites and predators community composition, agronomic and beekeeping management practices to be used in modelling. Representative means in this context that the selected scenarios should represent realistic condition for any physical sites known to exist in the assessment area for which information is available.

Any suitable functional form can be considered to describe the effect of a forcing variable on a target process. A useful solution that can be considered in many cases is to include the effect of the forcing variable into the function describing the target process. In this way the target process exists in two states, the normal state and the modified state in which the effect of the forcing variable is included.

For example, the basic (i.e., intrinsic) mortality function can be modified in the functional form adding a component that accounts for the mortality due to a biological agent. The mortality due to a biological agent can be modelled by a dose–response relationship describing how adult bee or juvenile stages are affected by a biological agent. We assume that dose–relationship is the same for all adults or all brood, so in the end, it is the exposure/dose that differs. The dose is the amount of agent (population abundance or prevalence) to which the colony is exposed. The response is bee stage-

specific and is given in terms of stage-specific mortality rate. The forcing variable is represented by the time variation of the population abundance or prevalence of the biological agent.

Further model developments could consider processes-based dynamical description of what has been considered only as a scenario (e.g. introducing a model for the population dynamics of the biological agents and their interaction with the honeybee colony population).

5.3. Model development, calibration and evaluation

Development, calibration and evaluation of a model are parts of a cyclical process whereby there is an initial focus on individual modules (code development, unit testing with respect to module behaviour against defined criteria, further code development and further unit testing). Once individual module testing proves satisfactory, modules are combined for further testing, including model calibration and evaluation, sensitivity analyses and post-model-development validation.

Before the computer model can be used as a regulatory model, it will need to be tested in at least three EU regulatory zones comprising different environmental scenarios, in terms of beekeeping and agronomic practices, abiotic parameters (climate and weather) and biotic parameters (e.g. resource availability and biological agents such as *Varroa destructor*, DWV, ABPV and *Nosema* spp.).

Post-model-development validation is reliant on field-data collection, which will not be completed for several years. As a consequence, model validation will be a lengthy process, conducted in stages. In the first stage, model calibration and evaluation will be conducted during model development, utilising published data and bee expert knowledge. Subsequently, once the field-data collection has been completed, post-model validation will be conducted using these data. Processes and parameters will also need to be revised, as a result of new published information and ad hoc data collection.

5.4. Future model expansion

The conceptual model developed by the working group is a first step. New opportunities for further development were identified by the working group, in particular in the following areas:

- The model is designed to assess risks to honeybee colonies from exposure to up to at least two substances (in mix tanks or from the parent compound and its metabolite). However, given the potential exposure from chemical mixtures and their potential interactions (synergism or antagonism), it is important that when the model is proved useful, it is further developed to include those types of interactions.
- The primary aim of the modelling tool is the risk assessment of pesticides. In addition, it should also prove useful for the assessment of mitigation measures to reduce risks from exposure to pesticides as seen in the recent study by Thorbek et al. (2016).
- The conceptual model for the module on Biological Agents could accommodate other biological agents and those could be added to the model as required.
- As a starting point, some of the modules of the model are applied as scenarios; however, further developments could consider processes-based dynamical description of what has been considered only as a scenario (e.g. introducing model for the population dynamics of the biological agents and their interaction with the bee population).
- The model currently considers a single colony; however, it could be expanded in time to consider multiple colonies to include potential infection/infestation transmission and spread among colonies at the apiary and region levels.

6. Foraging module

The foraging behaviour has links to the Colony module e.g. development of foragers, changing from nursing to foraging. Honey and pollen stores influence the decisions of foragers to rest or start foraging. The foraging behaviour is also closely linked to the food sources in the landscape (i.e. to distance to the hive, quantity of nectar, concentration of sugars in nectar and quantity and quality of pollen). Pesticides which affect the nervous system may affect not only the behaviour of foragers but also the behaviour of in-hive bees. As data are lacking, it is not possible to include all these effects in the model. Only effects on the foraging behaviour (e.g. homing behaviour; see also Section 9.5.2) should be taken into consideration in the Foraging module.

Foragers can collect pollen, nectar or water. The foraging activities (duration/handling time, distance, preference, etc.) are determined by the colony needs (brood development and proportion of nurses/brood), the environmental drivers (daylight, rainfall, etc.), the characteristics of the RPU (land cover, productivity in pollen/nectar, water availability, etc.).

6.1. Decisions of foragers

Foraging can be regarded as a sequence of decisions linking a sequence of behavioural elements. A schematic representation of the decision tree is reported in Figure 6.

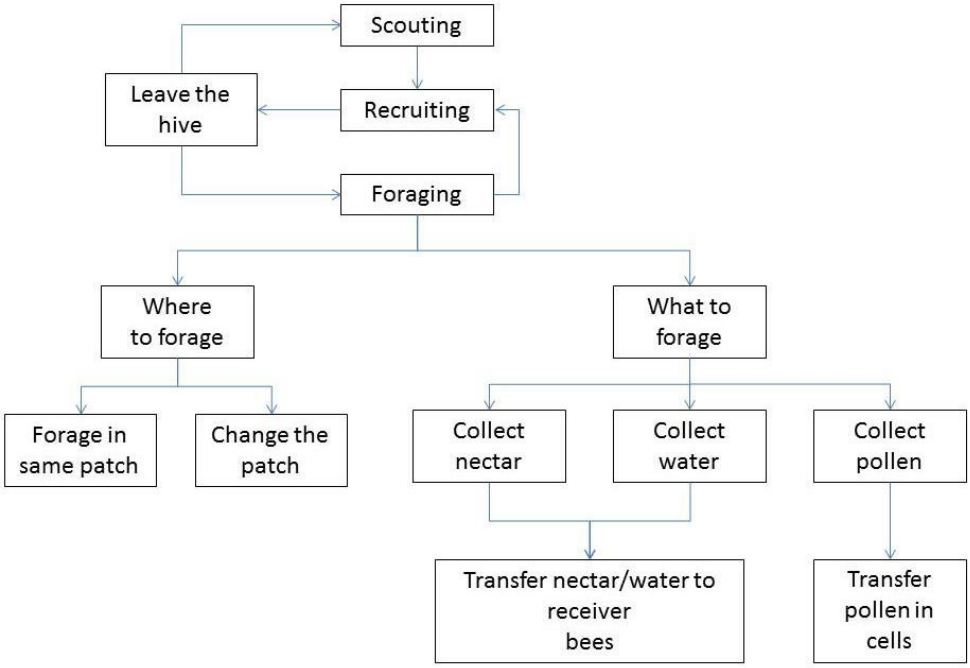

Figure 6: Decision tree for the foraging behaviour

6.1.1. Leave the hive or rest

Foragers leave the hive or rest. When a bee leaves the hive, it can be either a scout or a forager. The forager and the scout come back to the hive to recruit; recruited bees becoming foragers go to the same patch or to another.

The decision of a forager to leave the hive and start foraging is influenced by the following variables:

- Weather conditions (minimum and maximum temperatures, hours of daylight, wind speed and direction and rain).
- Nectar, pollen and water demands of the colony (size of the colony, e.g. brood reared/ space available for food storage).
- Overall maximum flight time per day.

6.1.2. Foraging nectar, pollen or water

There is a mechanism for decision making which is related to the demand in nectar, pollen and water and to the availability of the resource. The forager has three options:

- Continue to forage on the same patch.
- Go to a new patch advertised by another forager.
- Become a scout and search for a new patch.

The decision of a forager to become a scout is influenced by the following variables:

- Availability of food in the patches already visited (e.g. end of flowering period, diminishing of nectar).
- Quality of food in the patch already visited (e.g. lower sugar and protein concentrations for nectar and pollen, respectively).

The decision depends on the energetic efficacy related to nectar and pollen quantity and quality (e.g. amount of pollen collected within a given time and sugar content of the nectar collected) in the patch and their distance to the colony (Seeley et al., 1991; Seeley, 1995).

Foragers are informed on the quality of the nectar they bring to the hive by evaluating the time taken to unload the nectar in the hive. Foragers bringing back nectar with higher sugar concentrations are preferred by the receiver bees in the hive and can unload quicker than foragers bringing back nectar with lower sugar concentrations.

6.2. Behaviours of foragers

In the hive:

- resting,
- advertising a food patch (waggle dance),
- unloading nectar or pollen or water.

Outside the hive:

- flying to search for new nectar and pollen cell (see Section 8 for further details on 'cell') (e.g. guided search for a bee following the orientation provided by a recruiter or random for a scout bee),
- flying to a known nectar or pollen cells,
- collecting nectar or pollen,
- flying back to the hive,
- transfer of nectar to receiving bees and transfer of pollen in cells.

Each of these behaviours has a certain energetic requirement and risk of death. Energy requirement for resting is much lower than for foraging flights. The efficacy of foragers (in finding suitable resources and bringing high amounts within a given time) also increases with their age (Dukas and Visscher, 1994). Functions describing the different behavioural elements related to foraging need to consider also the environmental variables (e.g. hours of daylight, temperatures, rain and the resources in the RPU: e.g. amounts of nectar and pollen, sugar content in nectar and protein content in pollen) as well as the energetic and nutritional requirements of the colony.

7. Colony and in-Hive Products (C-HP) modules

7.1. Introduction

The key state variables of the C-HP modules are the colony size and the demographic structure (number of individual in the different biological or functional stages). We assume that the biomass for each adult stage is constant which allows the description of the population dynamics only in numbers. Finally, the other state variables are related to the in-hive products (food stores) that were categorised according to the way bees handle them (see Section 7.2 and Figure 10):

The honeybee colony can be thought of as a superorganism (social unit) with age-related division of labour and where all individuals rely on each other for the survival of the colony. Within a colony, during its lifespan, each bee performs a wide range of tasks, starting from the less complex task such as cleaning to the more complex ones such as foraging. In addition, depending on the needs of the colony, which relate to the colony age demography, there is behavioural plasticity.

An individual-based modelling approach is used to simulate the C-HP modules dynamics. In the model the concept of generalized individual is considered, which can be either a single biological individual (a bee) or a set of individuals of the same age (a cohort) and of the same biological responses. The model considers the following categories or stages of individual bees:

- The immature: eggs, larvae, pupae of workers and drones.
- The adults: the queen, the drones and workers (nurses, non-nurses, foragers and winter bees).

Figure 7 summarises the development of all individual bees (males and females) as well as temporal polyethism (shift between tasks in worker bees)

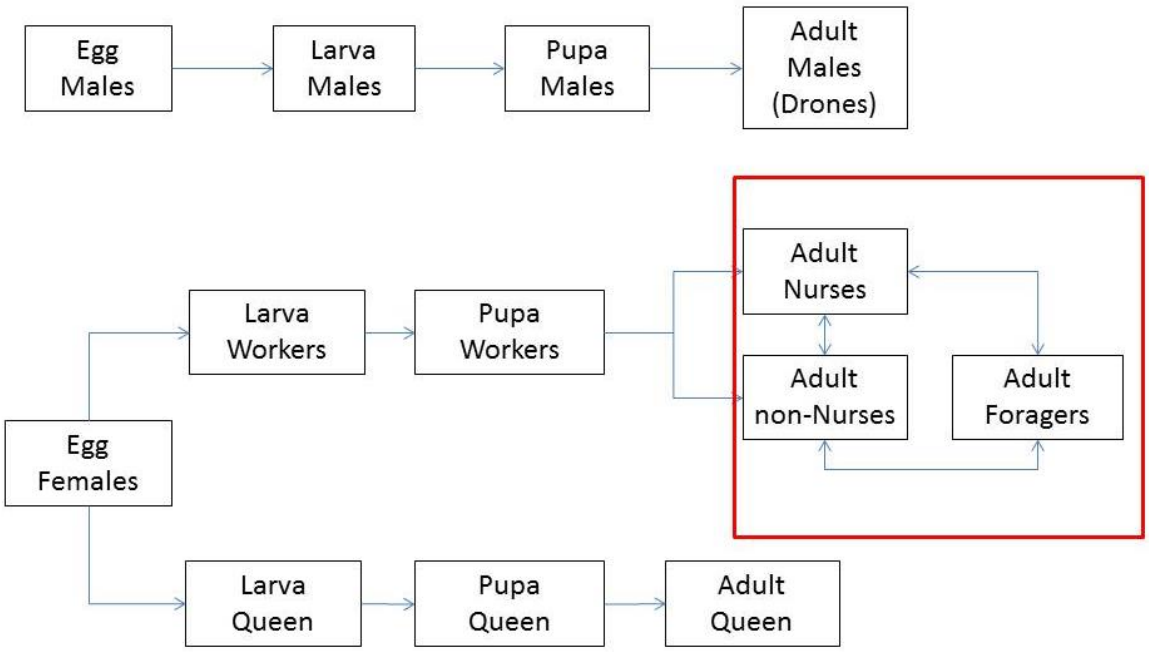

Figure 7: Demographic structure and transitions between summer worker bees

The time step for most of the biological processes is 1 day, although the time step for process simulation will be shorter when higher temporal resolution is required (e.g. 1 hour as for the main processes considered in the Pesticides module for outside the hive, as the contact exposure in the field, the flows of nectar, pollen and water).

Multiannual population dynamics of a honeybee colony have to be considered. Therefore, winter bees, which have a longer lifespan than summer bees, need to be included in the C-HP module.

Adult bees are classified in different functional stages according to their functional role in the colony on a given day:

For summer bees during spring, summer and autumn (corresponding to the 'active season' which varies across geographical regions):

- Nursing: this stage lasts for about 1 week (4–12 days) (Ribbands, 1953) and the task repertoire includes: visiting the brood cells, feeding the larvae with jelly (i.e. comprising royal jelly which is a proteinaceous substance secreted by the nurses' HPG and mandibular glands) and attending/caring the queen.
- Non-nursing: this group comprises in-hive bees aged, generally, from 12–21 days doing other tasks than nursing (i.e. cleaning the cells, cooling/heating the nest, receiving and storing food, producing wax and constructing combs, cleaning the debris and removing dead bees).
- Foraging: when about 21 days, bees become foragers and collect nectar, pollen, water, honeydew and propolis. Pollen and nectar make up most of the foraging activity except when weather is hot and where foragers collect water for cooling the colony. Within a given trip, foragers specialise on either pollen or nectar, but over their life, foragers are generalists.

For winter bees during winter:

- Nest thermoregulation (production of heat) for winter bees during winter (their tasks in mid to late winter switch to nursing and when conditions become favourable, they forage) (Figure 8).

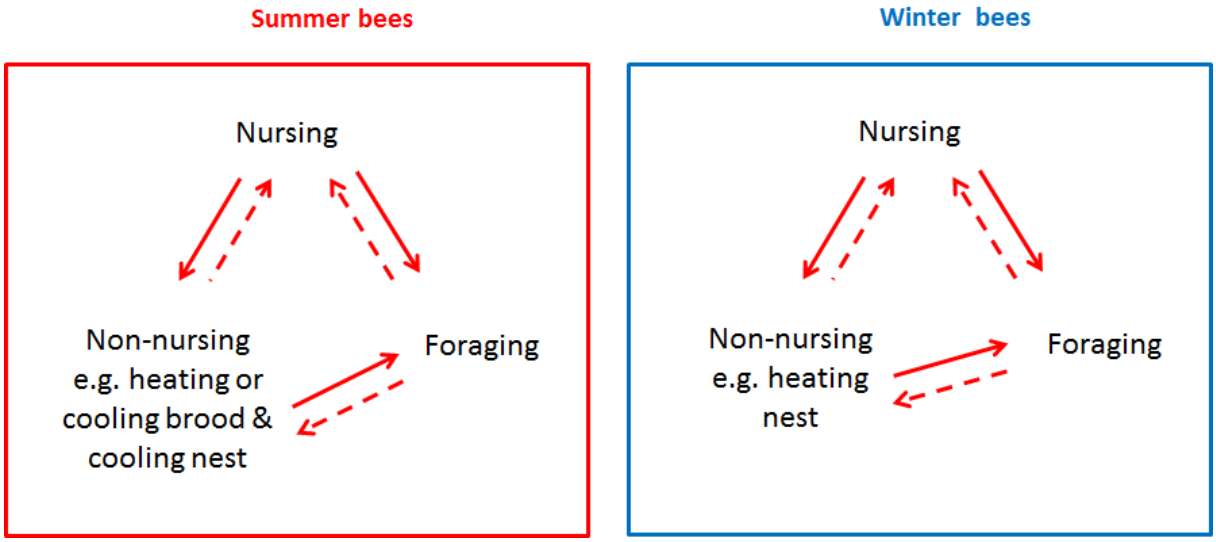

Figure 8: Developmental transitions of summer and winter bees

7.2. Conceptual models for the C-HP modules

The dynamics of the colony is described considering the bee life-history traits (Figure 9) such as:

- The demographic traits (egg-laying, mortality, development of brood and adults) (see Section 7.3.1).
- The behavioural traits (i.e. the ones that are critical to the colony development and maintenance: nursing, non-nursing, thermoregulating) (see Section 7.3.2); foraging is developed in Section 6.
- The physiological traits (e.g. energy requirements for each bee and susceptibility) traits (see Section 7.3.3).

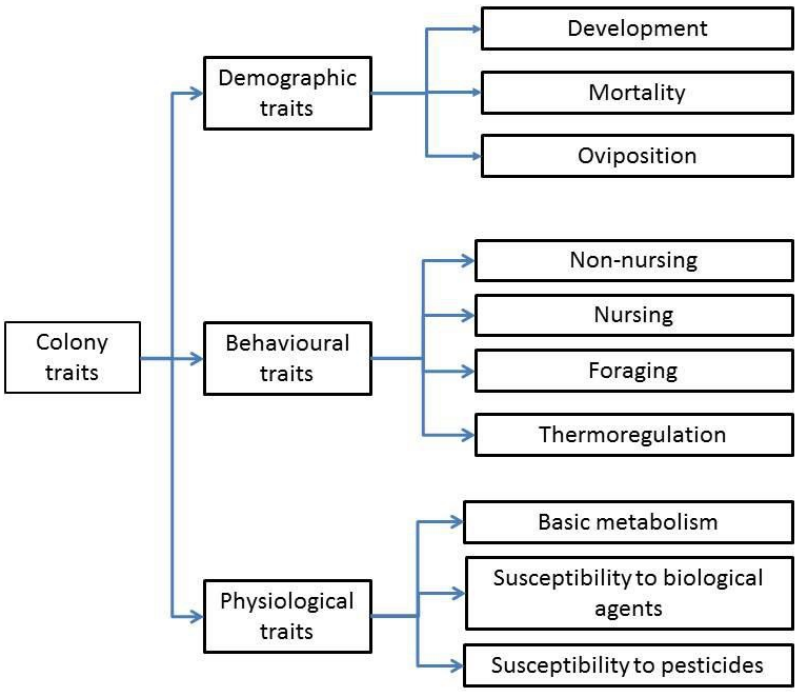

Figure 9: Conceptual model for the Colony module

The in-Hive Products module describes the process of production and consumption of:

- fresh pollen (stored pollen < 1 day old),
- beebread, (stored pollen > 1 day old),
- fresh nectar (nectar < 1 day old, i.e. either transported by foragers or stored in cells),
- freshly stored nectar (nectar stored in cells < 3 days old),
- honey (nectar stored in cells > 3 days old),
- jelly produced by nurses to feed larvae (which comprise royal jelly and other products; see below for more details)

In addition, non-nurse bees can produce wax and foragers can collect water, which is used by non-nurse bees during summer, to cool down the inside of the nest or to dilute honey.

The conceptual model for processing of nectar and pollen into food products is based on hourly flows of nectar and pollen as input. Processing of water is also based on an hourly basis. It seems sufficient to simulate the processing of nectar, pollen and water in the hive with a time step of 1 day because processing of nectar and pollen into food products requires one or several days and because the effect studies also are based on daily masses of pesticide consumed (see Table 5 and Section 9.5).

The nectar is either directly consumed or transformed into honey. The nectar is transformed into honey by evaporation of most of the water and conversion of the saccharose into fructose and glucose. To evaporate the water, the bees pump out the contents of their honey sac into a flat droplet on the underside of the proboscis and draw this droplet up again; this process is rapidly repeated for 15–20 min (Crane, 1975). Thus, the bees produce half-ripened honey containing 50–60% sugar. Then, the bees deposit the half-ripened honey in small droplets on the cell walls or in a thin film on the cell floor. This ripening takes 1–3 days. Ripened honey has a sugar content of 80% and thus a water content of 20%[6]. This transformation into honey is visualised in Figure 10 by the 'evaporation tank'.

Accordingly, the conceptual model distinguishes three compartments of stores referred to here as the 'fresh nectar', the 'freshly stored nectar' in the evaporation tank and the 'honey'. Honey can be consumed directly or diluted with water to be fed to the larvae.

The pollen cannot be consumed directly: it has to undergo some processing (visualised by the 'beebread preparation tank' in Figure 10). This takes at least 3 days and the result of the process is the beebread stored in beebread cells. For the beebread cells, daily cohorts of the cells that are filled on a certain day are needed because these are required for describing the consumption of pesticide present in the beebread (see Section 9.4).

Honey and beebread can be consumed directly by the bees but not by the larvae, only marginally (in relation to the total amount of protein necessary for complete larval development, the contribution of the protein by feeding larvae directly with pollen is less than 5% (Babendreier et al., 2004)).

Honey and beebread are processed by the nurses and added to the royal jelly to feed larvae (Figure 10). The royal jelly has two components: a substance from the mandibular glands ('white component') and from the HPG ('clear component') (Winston, 1987). The composition of the royal jelly is different for queens and workers. The queen larvae receives mostly 'white component' during the first 3 days, and a 1:1 ratio of 'white component' to 'clear component' during the last 2 days of feeding. For worker and drone larvae, during the first 3 days of their development, they consume only royal jelly but during the next 2 days a mixture of royal jelly with honey and beebread. The sugar content of the worker and drone larva food for the first 3 days is 5.4 and 2.9 mg respectively, and during the next 2 days, 54 and 144 mg, respectively (see EFSA PPR Panel, 2012 for more details on the calculations of the food eaten during the larvae development; Crailsheim et al., 2013; Mandla and Kumar, 2016).

[6] Sugar content = mass of sugar divided by mass of nectar; water content = mass of water divided by mass of nectar or honey.

While the amount of pollen in beebread consumed by older worker larvae is 1.5-2 mg, the amount of pollen in beebread consumed by drone larvae is not known (Babendreier et al., 2004).

The above food products (beebread, fresh nectar, stored fresh nectar, honey, honey diluted with water and jelly) consumed and/or handled by bees are summarised in the table below (Table 2).

Other matrices like honeydew or propolis may also be of interest. Therefore, it is recommended that the model is designed in a way that it can be expanded to integrate such parameters when more data become available in the future. As regards to honeydew, it may be considered in a similar way to nectar.

Table 2: Food products consumed (Co) and/or handled (Ha) by bees, according to their life stage

Stage\ products	Beebread	Fresh nectar	Stored fresh nectar	Honey	Honey diluted with water	Jelly
Larva	Co	-	Co	-	Co	Co
Nurse	Co	-	Co	Co	Ha	Ha
Non-nurse	Ha/Co	Ha[(a)]	H/C	Ha/Co	-	-
Forager	-	Ha/Co[(b)]	Co	Co	-	-
Drone	-	-	Co	Co	-	-
Queen	-	-		Co	-	Co
Winter bee	Ha/Co	Ha/Co	Ha/C	Ha/Co	Ha	Ha

(a) Receivers (in-hive bees) upload the incoming nectar from foragers.
(b) In cases of long-distance flights, foragers may consume some nectar they collected for their energetics demands.

For each compartment of the in-hive products, the inward and outward flux are described and linked to the processes responsible of the variation in the amounts of products stored (see Figure 10).

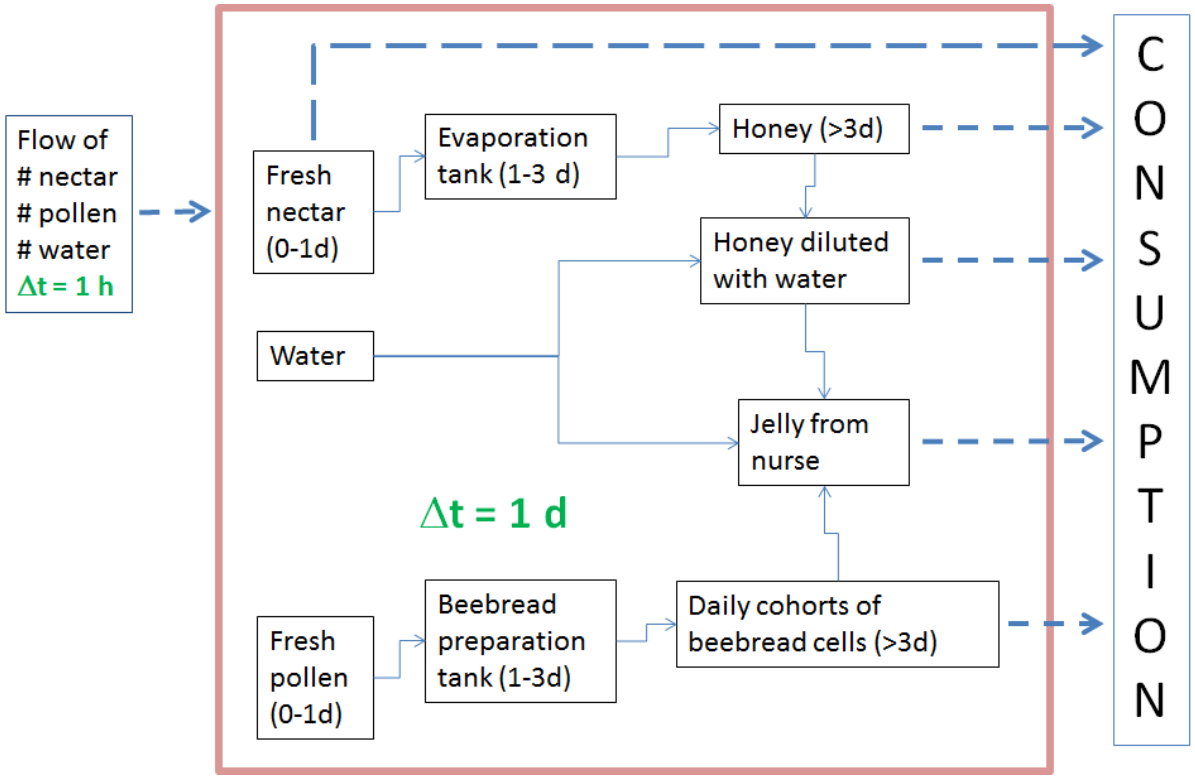

Figure 10: Conceptual model for the in-Hive Products module (processing of nectar, pollen and water into food products)

7.3. Colony module

The Colony module relies on a physiologically-based approach. The population dynamics of the colony are influenced by the ratio between the supply and demand for the basic resources. The availability of resources influences all of the basic rate functions that characterise the bee life-history traits. A minimum amount of pollen and nectar stored in combs will be required for the colony to start its development. Other stimuli influence the individual and 'collective' tactical and strategic activation and interruption of demographic, behavioural and physiological traits (e.g. the type, quality and distance of food sources activate and modulate the foraging activity). Also environmental driving variables (e.g. temperature) directly or indirectly have an influence on life-history traits. Finally, biological agents and pesticides are considered as stressors having an impact on the rate function defining the demographic (e.g. mortality), behavioural (e.g. onset of atypical or poorly effective behaviour) and physiological traits (e.g. susceptibility to other stressors) (Figure 11).

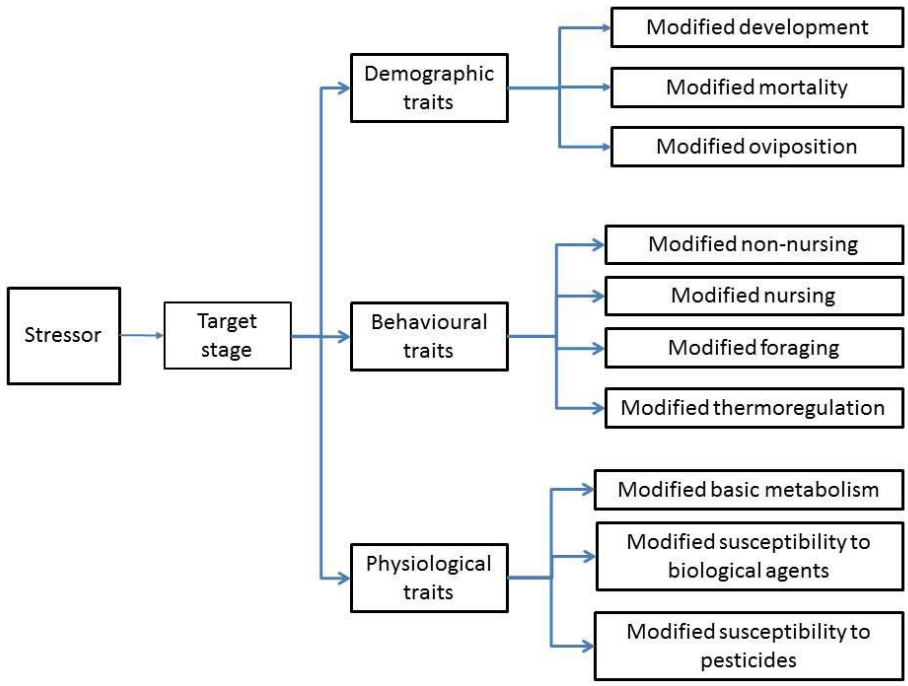

Figure 11: Conceptual model for the Colony module with effects from a stressor on colony traits

7.3.1. Demographic traits

The population abundance and stage-structure of the honeybee colony and their temporal dynamics are dependent on the initial conditions at the beginning of the simulation:

- Initial size and structure of the colony at start (after winter, the colony will contain a minimum number of 'n' worker bees in each functional stage from which the colony will start its development).

The demographic traits are responsible of the population dynamics and are described in terms of stage-specific rate function:

The **oviposition rate** (egg laying rate): number of drone and worker eggs laid by the queen in the time unit. Egg-laying rate is highly variable (1000 to 3000 eggs/day), varying mainly as a function of:

- colony population size (Al Ghamdi and Hoopingarner, 2004);
- queen age e.g. young queens of one or two years old are up to 30% more productive than older queens (Avetisyan, 1961; Woyke, 1984; Genç, 1992; Akyol et al., 2008). The proportion of drone eggs also increases with older queens as sperm number in the spermatheca decreases (Page, 1986; Szabo and Heikel, 1987);
- number of available brood cells in the brood chamber;
- time in the year (e.g.: onset of the oviposition of worker and/or drone eggs). Another example is seasonal variation for the oviposition rate in relation to climatic zones resulting in different amounts of brood (Hatjina et al., 2014);
- pollen availability (e.g. pollen collection and pollen stored in the colony) is correlated with the egg-laying rate of the queen (Cale et al., 1968; Al-Tikritya et al., 1972; Hellmich and Rothenbuhler 1986a,b; Weidenmüller and Tautz, 2002). Drones are also produced during a given time of the year and their production is linked to pollen availability (Czekońska et al., 2015). Ratio between nurses and the other categories of bees (non-nursing and foraging bees);
- in the presence of biotic (see Biological Agents module) and abiotic stressors (see Pesticides module), there is a modified mortality rate, different for each physiological stage.

The **stage-specific mortality rate** is composed of background mortality rate and additional mortality rate due to the effect of abiotic and biotic stressors (e.g. an increase mortality rate could be observed in larvae and adults infected by biological agents (see Biological Agents module) and/or exposed to pesticides (see Pesticides module) and BMP (e.g. supplementary feeding/introduction of a new queen). The basic mortality rate is influenced by:

- availability of food, especially proteins: e.g. cannibalism from workers may occur in eggs and larvae, going from earlier to older stages (1–4 days old) and result in increased mortality of drone and worker larvae(Schmickl and Crailsheim, 2007; Wharton et al., 2008);
- colony size. An increased mortality rate is observed in larvae when numbers of nurses are too low to feed the brood and maintain the nest temperature (Schmickl and Crailsheim, 2007; Torres et al., 2015).
- activity: e.g. number of hours of flight for the forager (the available hours for foraging are the result of a set of environmental conditions in each climatic or geographical zone (see Hatjina et al., 2014);
- in the presence of biotic (see Biological Agents module) and abiotic (see Pesticides module) stressors, there is a modified mortality rate, different for each physiological stage.

The **development** describes the change of the age in a stage and the transition from a stage to the following one. The age is measured in chronological time (days).

- **Development period for immatures** (drones and workers), from oviposition to adult emergence, is expressed in numbers of days from eggs to larvae, from larvae to pupae and from pupae until emergence. The development of immatures (drones and workers) is influenced mostly by food (pollen) availability and temperature (Winston, 1987; Medrzycki et al., 2010).
- **Development period for adults** or lifespan (drones and workers) describes the time required for the transition between different functional stages of the adult workers (and the reversibility in tasks between in-hive and foraging bees). It is expressed in number of days and it varies according to season (summer, spring/autumn and winter).
- There is also a modified development period for both adults and immatures as an effect of abiotic stressors (see Pesticides module) and biotic stressors (see Biological Agents module).

7.3.2. Behavioural traits

A behavioural trait is composed of a repertoire of tasks that can be grouped because they have the same functional meaning (for examples, the behavioural trait 'nursing' includes the following repertoire: warming and feeding the brood). When needed, the details for each behavioural trait are described.

For each behavioural trait, in standard conditions, the following is required:

- Regulation: starting and stopping age, triggering factors, timing (duration, frequency), efficiency (Winston, 1987).
- Demands in sugar, protein and water.

In the presence of biotic (biological agents) and abiotic (pesticides) stressors, the behavioural traits are modified in terms of:

- regulation: starting and stopping age, triggering factors, timing (duration, frequency), efficiency;
- behavioural efficiency: e.g. ability for an in-hive bee to clean cells in an efficient way (i.e. hygienic behaviour).

7.3.3. Physiological traits

For risk assessment modelling purposes, the degree of gland development, or quantity and quality of other tissue and pheromone production were not considered as physiological traits, due to the lack of

adequate data which correlates them with the food quantity and quality, and the difficulties in measuring the effects of the stressors on these traits. On the contrary, the energy expenditure, as a measurement of the metabolism and the susceptibility to stressors were considered. In particular, for each physiological trait, in standard conditions, the following information is required:

- Basic metabolism: energetic expenditure for bees being inactive (i.e. resting).
- Additional demands in sugars, proteins and water for performing each demographic trait and behaviour.

In the presence of biotic (biological agents) and abiotic (pesticides) stressors, the physiological traits are modified in terms of:

- change in demands in sugars, proteins and water;
- susceptibility to biological agents;
- susceptibility to pesticides.

7.3.4. Conceptual model for consumption of nectar and food products

Within a given day, the consumption needs of the different categories of bees are calculated and these are the basis of the calculation of the required consumption of nectar/honey, beebread at the colony level.

Within a given day, the bees consume first the freshly stored nectar from the evaporation tank, and if this is not sufficient, they consume honey (capped nectar after uncapping it) (Figure 10).

The conceptual model for the consumption of beebread needs to be more detailed because of the experience that bees prefer to have filled beebread cells in close proximity to the brood. Furthermore, they prefer consumption of beebread that is stored for less than 3 days (Anderson et al., 2014). This has the consequence that there is preferential filling and emptying of beebread cells close to the brood. It is important that this is simulated because pesticide concentrations in the pollen may vary considerably from day to day.

A hive needs several tens of kilos of pollen per year, ranging from a few tens to 55 kg (Louveaux, 1968; Seeley, 1985; Winston, 1987, Liolios et al., 2016). However, it has been documented that the number of cells filled with pollen is related to the number of brood cells (maybe the number of adult bees as well) (e.g. the ratio of pollen cells to the brood cells was found to be between 0.2 and 0.4, in an experimental set up in South Europe (Hatjina et al., 2013, unpublished set of data), which could be one of the lowests in Europe.

The possible number of cells in the colony could then be devided into sets/classes of 1000 cells. Class 1 is assume to be closest to the brood and class 5, 6, 7, or 8 is assumed to be at the farther distance of the brood The priority of filling the cells with beebread on a certain day is that first all empty cells of class 1 are filled, then the empty cells of class 2 and so on.

The priority of emptying the beebread cells on a certain day is as follows:
first all cells of class 1 are emptied with preference for the cells that had the shortest storage times, not including the 1–3 days storage time needed to process the pollen ('beebread preparation tank' in Figure 10)
then all cells of class 2 are emptied with preference for the cells that had the shortest storage times, not including the 1–3 days storage time needed to process the pollen ('beebread preparation tank' in Figure 10)
then the cells of the classes 3, 4, 5 and 6 are emptied subsequently, following the same procedure as described above for the classes 1 and 2.

This procedure has the consequence that for each individual beebread cell in each class the following state variables have to be simulated:
is the cell empty or filled, yes or no?
what is the age of the beebread in the cell?

If simulations show that 6000 beebread cells are not enough, then more classes (of 1000 cells each) can be added as needed following the same procedure as described above.

8. Resource Providing Unit and Environmental Drivers (RPU-ED) modules

It is anticipated that the model will be suitable for high tier risk assessment. Therefore, an RPU/ED module will be needed in the final model, representing the resource-providing unit that surrounds the modelled colony, all relevant environmental drivers and other data relevant to foraging. The concept of the Service Providing Unit (SPU) (Luck et al., 2003) highlights links between populations of species and ecosystem services and consequences of changes in population characteristics on service provisioning. This concept was further used for environmental risk assessment purposes (Gilioli et al., 2014). The RPU is derived from the SPU concept. It includes both abiotic and biotic factors of the environment (including predators). However, in the RPU-ED modules, predators are not considered.

Intermediate and final model outputs can be directly compared with detailed spatial and temporal field data from the RPU where all data need to be collected to calibrate and evaluate the model.

8.1. Module State Variables

The key state variables from the RPU/ED module will be directly relevant to area, patch and foraging behaviour and will include:

- weather: temperature, relative humidity, wind direction and speed (see also Section 9.2.2 for more details), total precipitation and solar radiation;
- spatial landscape information (the location, shape and area of all relevant landscape structures (i.e. the spatial landscape as a series of unique habitats or 'patches');
- location of colony and patch and derived distances;
- the availability of pollen, nectar and water, including the quantity (mg) of pollen, the protein content of pollen (w/w %), the quantity (mg) of nectar, the sugar content (w/w %) of nectar, the presence (y/n) of water;
- pesticide contamination in space and time in pollen, nectar and water (see Section 9.2.3).

Patch-level factors in addition to distance from the hive or availability of pollen, nectar and water that may also influence foraging decisions. Therefore, the key parameters will all input directly into the Foraging module.

The RPU-ED modules will need to cover the RPU, covering an area with a radius of 3 km around each modelled colony. The required level of spatial precision is 1 m^2 (noting the importance of structures such as field margins in relation to key module outputs), however, it is suggested that this could be achieved as a two-step approach: through data collected at the RPU, patch and cell levels for the landscape structure and through data collected at the level of the RPU for landscape dynamics.

The RPU-ED modules should be able to handle hourly data for all time-varying parameters relevant to key model output parameters.

8.2. Landscape and environmental characteristics

The landscape in the RPU-ED modules is hierarchically structured as: RPU (3 km radius - Heterogenous ~ plant phenology) > Patch (Homogeneous ~ plant phenology) > Cell (technical resolution: 1 m^2).

8.2.1. Landscape structure

Relevant to [a] pollen, nectar and water availability, [b] pesticide contamination, and [c] foraging behaviour(Henry et al., 2014; Odoux et al., 2015; Sponsler and Johnson, 2015).

* At the level of the RPU:

- land-use type,

- vegetation:
    - arable/tilled areas (type of crop),
    - pasture,
    - wild flower meadows,
    - large trees.
- non-vegetated areas,
- soil type and associated characteristics.

* at the level of the patch:

- Polygon characteristics: a central location, shape and surface (with indications of distance from treated field for calculation of spray/dust drift with distance from application).
- Contents: fields (polygons with boundaries, shapes, sizes) and landmarks/barriers (vectors).

* At the level of the cell:

- The location of the colony will be known within 1 single cell.

8.2.2. Landscape dynamics

Relevant to [a] pollen, nectar and water availability and [b] pesticide contamination (hourly data for each patch):

- Plant phenology (existing dynamic models for plant growth and flowering): ignoring soil and environmental variables.
- Farm management events: planting (i.e. crop rotation), fertilizing, spraying, harvesting, ploughing.
- Plant pest control: how (method of application, e.g. spray, granules, seed), where, when.

Environmental drivers (relevant to [a] pollen, nectar and water availability, [b] pesticide contamination and [c] foraging behaviour) (hourly data for each RPU):

- Weather:
    - temperature,
    - relative humidity,
    - solar radiation,
    - total precipitation,
    - wind (direction and speed).
- Climate:
    - type (thermal sums, average temperature, average precipitation),
    - snow cover.

The RPU-ED modules should be able to handle the above data. Decisions concerning the scope and spatio-temporal precision of input data should be made in the context of the scope and spatio-temporal precision of the module outputs that are required.

9. Pesticides module

9.1. Introduction

This section describes all concepts that deal with pesticide exposure and effects. This is a combination of the conceptual models (i) for the exposure to pesticides in the landscape, (ii) for the in-hive exposure of the different physiological stages of bees to the pesticides, and (iii) for the effects of

pesticides on these stages of bees resulting from both in-hive exposure and from exposure in the field. The conceptual model for these effects (shown in Figure 12) shows that the Pesticides module assumes that the following information is delivered to the module at a time scale of 1 h: (i) contact exposure in the field, (ii) flows of nectar, pollen and water, (iii) concentrations of the pesticide in this nectar, pollen and water. This time scale of hours is needed because e.g. pesticide concentrations in nectar may change drastically during the day after spray applications (EFSA PPR Panel, 2012, p. 175). The water may be surface water, water from puddles or guttation water (EFSA, 2014). Figure 12 shows furthermore that the effect module requires the exposure to nectar consumption during flying as an input.

The following bees are considered in the effect model (see Figure 12) larvae, pupae, nurse bees and other in-hive bees (referred as non-nurse bees in the Colony module) foragers, the queen and drones (it is assumed that the eggs are not exposed and therefore that there is no effect on them). Because the sensitivity of winter bees may be different from the sensitivity of summer bees, the effect module will distinguish between summer and winter bees.

With respect to contact exposure, there is the complication that this either may occur for foragers in the field, but also for the larvae which both consume the liquid phase of their food but also are in contact with the surface of the brood comb and are exposed to the pesticide in both ways (oral and contact).

The Pesticides module has to be able to simulate the in-hive exposure and effects of at least two substances (e.g. two pesticides sprayed in the same tank mix or one parent substance and a metabolite). Simulation of the formation of the metabolite in the hive should not be part of the module.

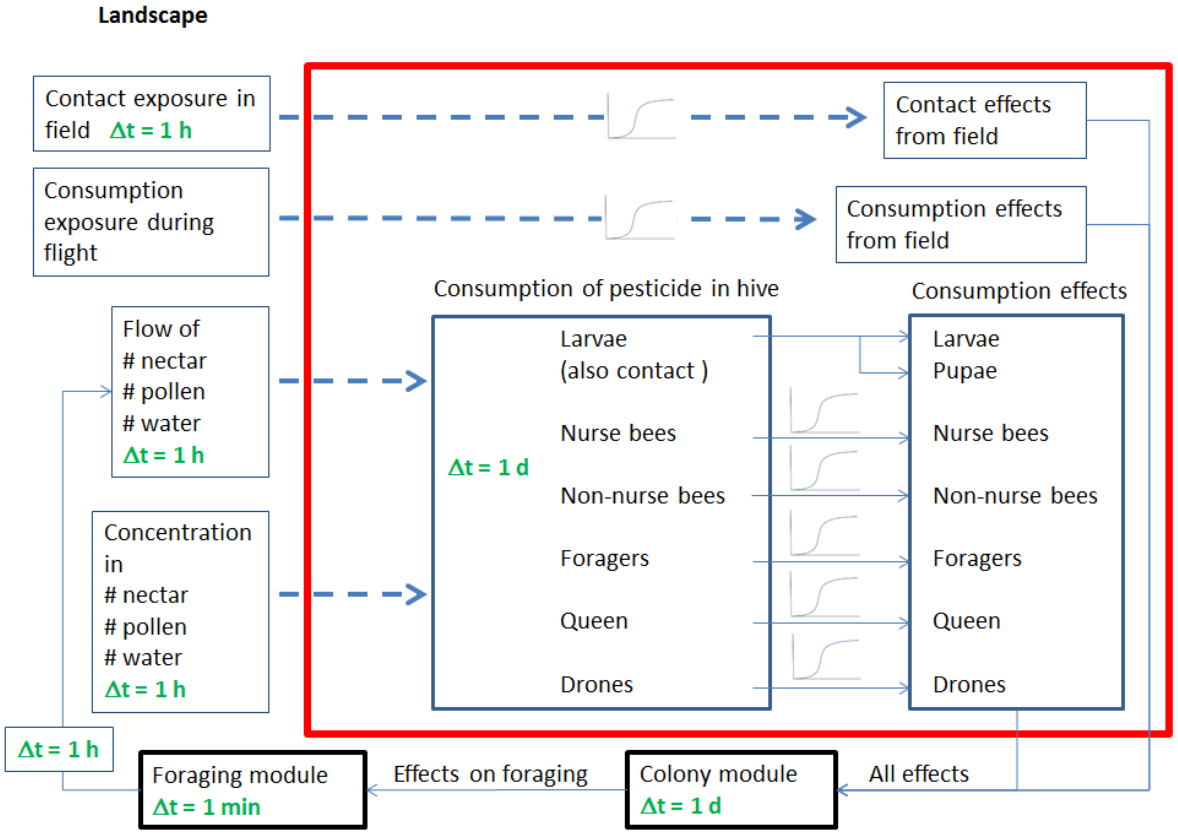

Figure 12: Overview of the conceptual model of the effects of pesticides on the different bees

The description of the pesticide consumption is based on four conceptual processes as shown in Figure 13.

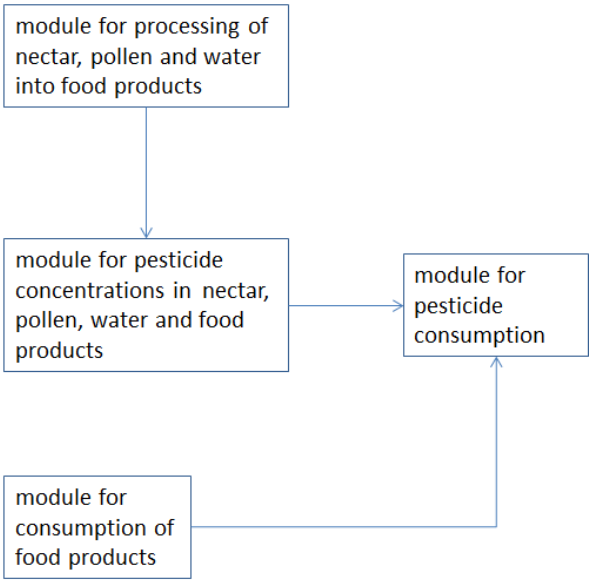

Figure 13: Schematic description of the conceptual processes required for assessment of pesticide consumption in the hive

The mechanisms for the processing of nectar, pollen and water into food products and for the consumption of food products have been described in Section 7.2. Thus, the following sections deal with pesticide exposure in the landscape, pesticide consumption and pesticide effects (the section on the effects will include the linking concepts between pesticide consumption and exposure, Table 4 in Section 9.4). For an overview of the state variables (i) required as input to the Pesticides module and (ii) described by the Pesticides module, respectively.

9.2. Exposure to pesticides in the landscape

9.2.1. Introduction

The forager is the one with direct contact to pesticides in the landscape. Two main routes of exposure should be distinguished which will be dealt with in the following sections:

- contact exposure (this section will describe how the magnitude of the contact exposure should be modelled),
- oral exposure.

The concentrations of the bee relevant matrixes are relevant for the whole colony (i.e. for all bees) as the foragers collect and bring back those materials with the pesticides into the hives. The following matrixes should be considered (as the most important ones):

- pollen,
- nectar,
- water (guttation fluid, puddle water and surface water).

The section on oral exposure will describe how the concentrations of the bee relevant matrixes in the landscape should be determined. This information can be used in a next step to simulate the residues entering the hive.

9.2.2. Contact exposure

Forager bees can be contaminated to pesticides by visiting the treated field or visiting neighbouring off-field areas, which had been indirectly contaminated. It is considered that bees will be exposed to the pesticides only if there is flowering crop or there are flowering weeds/plants in those patches at the time of the pesticide application. Dynamic information on the crop status (i.e. BBCH grows stage,

if there is pollen or nectar available) is part of the RPU-ED modules (see Section 8). No contact exposure should be possible from those patches which do not offer food to the bees at the time of the application as bees will not visit those areas (insignificant visit, however, may not be excluded). The exposure from these patches can be neglected. Focussing on the treated field and the neighbouring patches offering food at the time of the application, the model should be able to perform the following procedure:

- The exact time (hour) of the pesticide application(s), the application method (downward or upward spraying, granular application, seed treatment), the application rate(s) (expressed as mass/area, e.g. kg/ha) and the interval between the applications (in case of multiple applications) should be user inputs.
- The model should inform the user in which BBCH stage(s)the treated crop is on the chosen date(s) and what is/are the actual weather condition(s) in the chosen hour(s) (temperature, rain and wind speed and direction), including a warning message if the weather conditions in the application hour are unfavourable for spray applications.
- Identification of those patches which offer food to the bees at the time of the application(s) (distance of the colony, type of food provided, size of patch).
- Identification of the number of bees foraging on the relevant patches at the time of the pesticide application(s) (hour). This is available from the Foraging module.
- If there are no bees in any of the relevant patches (e.g. evening application outside of the forager activity), the model should consider no contact exposure. Otherwise, the model should continue the procedure.
- Identification of the exposure level of the foragers: in accordance with EFSA (2013), the contact exposure of the foragers is in direct relation to the application rate. However, it is considered that not all individuals are exposed to the same level.

It is proposed that the cumulative exposure percentile (Fe) is taken into consideration using equation M8 on page 172 of EFSA, 2013b. With rearrangement of this equation, the cumulative mass (m) of the chemical deposited to the individual bees can be calculated as:

$$m = \frac{Fe * fdep * A}{10^1 * E}$$

Where:

- *m* is mass of the chemical deposited/bee in µg,
- *Fe* is the cumulative exposure probability (%),
- *fdep* is the deposition factor,
- *A* is the application rate in kg/ha,
- *E* is the estimated linear slope constant (-).

Ideally, the cumulative probability density function of *m* should originate from representative measurements. If no suitable information is available then the worst-case default values from EFSA (2013b) may be used (*E* = 2 for upward spraying to the treated crop and *E* = 1 for downward spraying to the treated crop and for spray drift and dust drift).

The default *fdep* for the treated crop is 1 for spray application (i.e. the treated field is contaminated by 100% of the application rate). However, for granular application after the emergence of the crop, it should be equal to the released chemical in the air during the application (this depends on the dust formation, dust release and the chemical content of the released dust). This should be expressed as fraction of the application rate and should be a user input. If this is not known, a default *fdep* value of 0.1, as recommended by EFSA (2013b), may be used (i.e. 10% of the chemical is realised to the air by the dust during broadcasted application of granules). For seed treatment and granular application before the emergence this factor is not relevant (i.e. *fdep* = 0) as the crop cannot be in flowering stage.

As regards weeds present in the treated field, the *fdep* values are related to the crop interception. Default *fdep* values for different crop phenology (i.e. BBCH stage) of different crops from Table X2 of EFSA (2013b) should be considered.

As regards deposition to the off-field areas, the model should offer two options: (i) fixed user-defined *fdep* values for the different off-field areas (field margins, adjacent crops), and (ii) description of deposition of spray or dust drift as a function of downwind distance to the treated field with the two sequential power functions described by the equation (Eqn) 2 on p. 116 of FOCUS (2001) with the user-defined input parameters *A*, *B*, *C*, *D* and *H* as defined in this Eqn 2.

For option (ii):

- Tentative guesses for these *A*, *B*, *C*, *D* and *H* parameter values can be found in Appendix B of FOCUS (2001) for spray drift deposition for a range of crops (crop categories). In case of dust drift, such tentative guesses are not available; it is thus necessary to measure these parameters by dust drift deposition measurements as a function of distance to the treated field downwind in the wind direction before running the model. For dust drift deposition in in-field and off-field areas, the model should estimate dust deposition including an increase factor accounting for the fact that flowers catch more dust than is measured in dust deposition experiments on the soil surface (EFSA, 2013b, p. 140). This increase factor should be an input parameter of the model.
- The model should use hourly average measurements of the wind direction and simulate the effect of this wind direction by dividing the distance to the treated field by the cosinus of the angle of the wind direction as described by van de Zande et al. (2012, p. 24).
- The model should furthermore calculate the average deposition onto 1-m wide strips of the off-field areas using Eqn 4 on p. 117 of FOCUS (2001). These 1-m wide strips should be used because averaging the deposition over e.g. the full width of the adjacent field will lead to a large dilution of the exposure concentrations which is not appropriate.

9.2.3. Concentrations of pesticides in nectar, pollen and water

In patches

Forager bees can collect items contaminated with pesticides by visiting the treated field or visiting neighbouring off-field areas, which had been indirectly contaminated. Since the pesticide concentrations are highly variable in space and time in a real landscape, the information used by the model should also be dynamic. Spatially patch level description is sufficient. From temporal point of view, hourly data should be used. It is noted, that this time resolution will be necessary only for modelling spray applications during the flowering period, as the concentrations in pollen and nectar can dramatically change in a short time (EFSA PPR Panel, 2012). For spray applications outside of the flowering period or for solid applications, less frequent time steps (e.g. days) are also considered sufficient.

Information from the landscape composition is available from the RPU-ED modules. The RPU-ED modules contain quantitative and dynamic description of relevant matrixes occurring on the landscape. The following types of matrixes and patches should be distinguished:

- Treated field: pollen and nectar of the treated crop, pollen and nectar of the weeds in the field, guttation fluid from the crop, puddles.
- Indirectly contaminated area: pollen and nectar of crops from cultivated fields, pollen and nectar of plants from vegetative field margins, water bodies.

Spatial variability of the concentrations in the patches

The contamination level of the bee relevant matrixes (pollen, nectar, water) are related to the use of the pesticide; most importantly the application method, application time(s) and frequency, the application rate(s), the crop type what is treated; crop and plant types available in the vicinity, the physical-chemical properties of the pesticide and some abiotic environmental parameters like soil properties (where considerable degradation/dissipation may happen), dimensions of the water bodies or wind speed and direction (during the applications). Since there is a large number of possible combination of the driving factors, we recommend do not model these concentrations for the treated crop(s) within the (population) model. Rather, the concentrations of the treated crop in each individual treated field in the landscape should be user defined input parameters for the model (i.e. a

separate input field with information grids for each treated field of the selected landscape). This means that information/estimation on residue levels of the relevant matrixes should be available before running the model. The use of worst-case default values may also be eligible (see EFSA, 2013b). If no suitable information is available on the temporal variability of the residues, then the peak concentrations belonging to each application (in case of multiple applications) should be used as input. We recommend insert these concentrations into the model expressed as residue unit dose (*RUD*) values. *RUD* is the residue unit dose calculated for application rate of 1 kg/ha and for 1 mg/seed, where applicable. The application rate expressed as mass/area (kg/ha) and mass/seed (mg/seed) should also be user inputs. The model should calculate the actual predicted environmental concentrations (PEC) in the matrixes for the treated fields considering this information using the following formula:

$$PEC = RUD * A$$

Where:

- *PEC* is the predicted environmental concentration (mg/kg) – it is noted that in certain context this *PEC* may be based on measurements,
- *RUD* is the residue unit dose, defined as the residue in mg/kg at a dose of 1 kg/ha or 1 mg/seed,
- *A* is the application rate (kg/ha or 1 mg/seed).

The application rate expressed as mass/seed is relevant only for the concentrations appearing in pollen and nectar of the treated crop after seed treatment application. For all other cases, the application rate expressed as mass/area is the relevant quantity.

Ideally, the residue levels of the relevant matrixes of all other crops and plants originate from representative measurements. However, it is not expected that such a large number of measurements (with sufficiently low level of detection/level of quantification LOQ/LOD) will often be available (in a complex landscape, there could be > 100 relevant patches potentially visited by bees). Therefore, the model should be able to estimate (model) those concentrations using the information that is available (i.e. *RUD*s established for the treated crop(s) will always be available and some additional measurements may be required for the modelling exercise). A separate input field should be available for those concentrations (pollen and nectar concentrations in crops/plants other than the treated crop).

As regards to weeds present in the treated field, concentrations in pollen and nectar could be estimated (modelled) by taking into consideration the crop interception. Default *fdep* values for the deposition to weeds in different crops and crop stages are available in Appendix X of EFSA (2013b).

For the concentrations in the matrices in the off-field areas, the same approach should be followed as for the contact exposure as described before. Therefore, the concentrations should be multiplied with *fdep* and the same two options should be offered for the assessment of the *fdep*. However, the deposition should not be calculated for 1-m wide strips, but instead averaged over the full width of the off-field area (because these concentrations are averaged anyhow after entering the hive).

There are still some doubts whether the linear proportionality of the *PEC* to the application rate is also valid for seed treatments. Nevertheless (in the absence of better alternatives), it is recommended to use this linear proportionality.

Temporal variability

Information on the residue behaviour in the matrix could help to decide whether the peak concentrations should be used as input or a kind of average concentration in time. The latter is when – based on existing knowledge – it is expected that concentration in pollen and nectar do not vary considerable during the flowering period. This may be expected for pre-sowing soil applications for the treated crop. If this is the case, the inserted values are considered to represent the time variability of the residues for that patch.

Concentration in water is usually expected to not show stability in time. Concentrations in pollen and nectar after a spray application made during the flowering period are expected to undergo in a fast drop down. Similarly, a rather fast dissipation/degradation is expected from the pollen and nectar of

field margin plants or adjacent crops indirectly contaminated (i.e. spray drift or dust drift). In case of multiple applications, next to the dissipation/degradation processes, accumulation may also happen resulting in multiple peaks with different height.

These processes should be studied and quantitatively described before running the model. In case of good understanding of the residue pattern, these quantitative data should directly be inserted into the input field of the Pesticides module. In case of limited knowledge on the residue pattern, recommendations of EFSA (2013b), may be considered (e.g. concentration patterns in surface water and puddle water can be modelled, a screening level exposure estimation, but also refinement options are available for guttation fluid, considerations for spray drift, dust drift or crop interception are available).
As regards the dissipation from pollen and nectar, as well as from water, we recommend that the model should be able to estimate the residue decline following first-order kinetics. For this, the dissipation rate constant or the dissipation half-life (DT_{50}) should be the input parameter. The first-order kinetics can be modelled by the following equation:

$DT_{50} = \ln(2) / k$ or $k = \ln(2) / DT_{50}$

Where:

- DT_{50} is the dissipation half-life in the given matrix in days (time taken for 50% of the chemical to disappear from a compartment by dissipation/degradation processes),
- k is the rate constant (d^{-1}).

For the different types of water, the dissipation/degradation half-live may be estimated from information in pesticide dossiers (otherwise no degradation may be considered). However, such information may not be available for pollen and nectar. In case where the dissipation processes had not been studied for pollen and nectar (i.e. DT_{50} or k is not known), it is recommended to consider the following worst-case procedure (also considered by EFSA, 2013b):

- Exposure due to spray liquid during the flowering: DT_{50} = 10 days or k = 0.0693 d^{-1}.
- All other cases: practically no degradation; i.e. DT_{50} = 1000 days or k =0.000693 d^{-1}.

This is recommended equally for crops/plants in the treated field or for crops/plants in indirectly contaminated areas.

Nevertheless, the model should be able to consider other types of kinetics e.g. *via* direct insertion of the complex pattern into the input field of the Pesticides module for each patch of the selected landscape for each time step (i.e. hours). This option maybe used in cases where the dissipation/degradation processes had been studied and it is justified that the process does not follow first-order kinetics. For example, it may be expected that the residue decline after the contamination to spray liquid during the flowering will initially be very fast followed by a slower phase of dissipation/degradation.

In summary, the model should be able to consider two ways as possible inputs for pesticide concentrations:

- The peak concentrations are available and it is expected that the residues behaving the same way after each application: the peak concentrations in time belonging to each application for each patch are input parameters (provided by the user). The model describes the residue decline in each patch considering a first-order kinetic after each application (after each peak). Alternatively, a time representative concentration maybe used considering no degradation (only if temporal variability is not expected; for more explanations see above).
- Complex residues behaviour is expected and/or different kinetics from first-order is to be modelled: the concentrations for each patch and each time step (24 hours a day) should be inserted into the input field of the Pesticides module by the user.

Entering the hive

The model should calculate the mass of the residues entering the hive by combining the pesticide concentrations at the patches and the collected food from each patch. This computation should be

done on hourly basis and separately for pollen, nectar and water. The daily average concentrations entering the hive should be calculated as a next step by considering the hourly collected food or water and their concentrations (Tables 3 and 4). These daily average concentrations will be used further in the Pesticides module.

Table 3: Overview of input into and output from the landscape pesticide exposure module

What this section needs (most of them on hourly basis)	From which module(s)	What this module provides
Temperature	RPU-ED	Mass attached to forager bees
Rain	RPU-ED	Pesticide concentration in nectar (at patch and entering the hive)
Wind speed	RPU-ED	Pesticide concentration in pollen (at patch and entering the hive)
Wind direction	RPU-ED	Pesticide concentration in guttation fluid (at patch and entering the hive)
Nectar availability	RPU-ED	Pesticide concentration in puddle water (at patch and entering the hive)
Pollen availability	RPU-ED	Pesticide concentration in surface water (at patch and entering the hive)
Guttation fluid availability	RPU-ED	
Puddle water availability	RPU-ED	
Surface water availability	RPU-ED	
Crop type and phenology of the crop (BBCH)	RPU-ED	
Width of the patch	RPU-ED	
No. of foragers on each patch	Foraging	
Collected food from each patch	Foraging	

9.3. Conceptual model for pesticides concentrations in water, food products and larvae cells

For the module on pesticides effects, the following types of pesticide concentrations are needed (daily values):

concentration in the nectar evaporation tank;

concentration in the water;

concentration in the honey;

concentration in the beebread preparation tank and beebread cells;

concentration in the jelly;

concentration in the larvae cells.

The concentrations in the nectar and water are hourly values delivered to the Pesticides module (Figure 12) and these have to be transferred into daily average values by summing up the pesticide masses in the hourly flows and dividing the total pesticide mass by the total mass of nectar or water.

Figure 14 visualises the conceptual model for the concentrations in the beebread, honey, jelly and larvae cells.

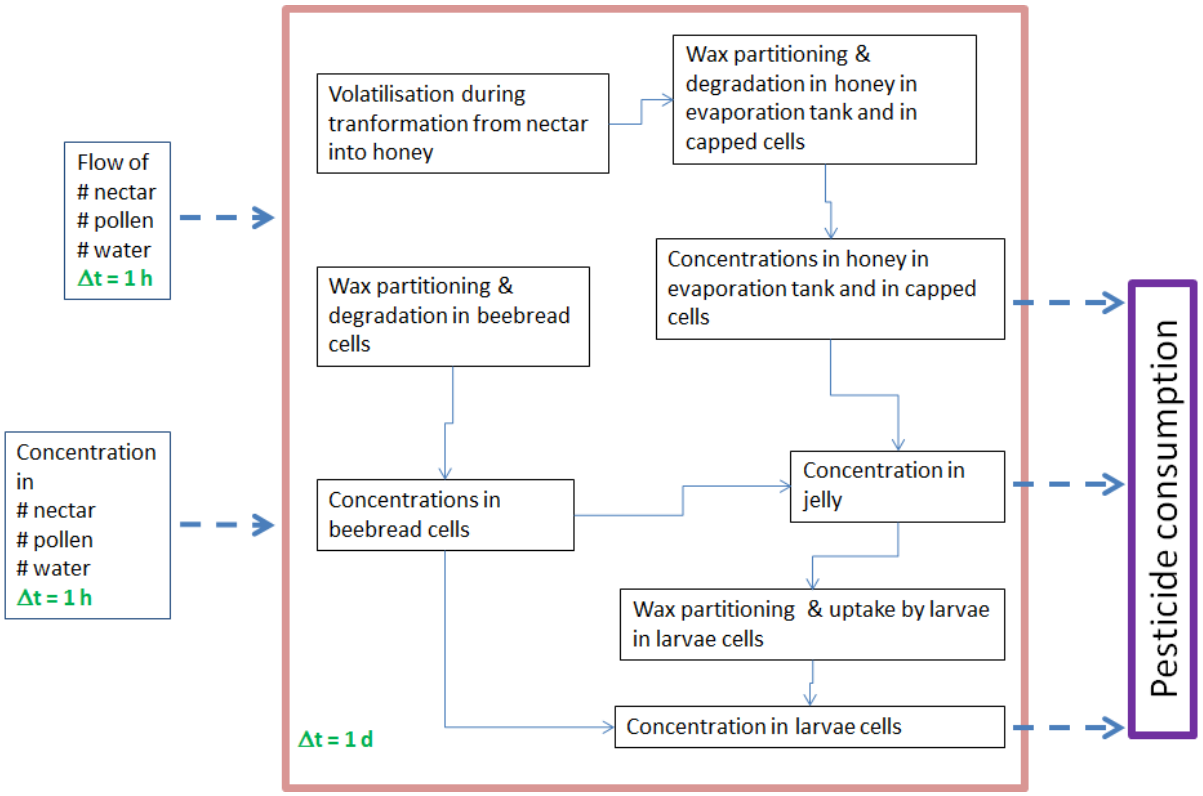

Figure 14: Overview of the conceptual module for the pesticide concentrations in food products and in larvae cells

First, the concepts for the concentrations in the honey are described, followed by those for the concentrations in the beebread cells, the jelly and the larvae cells.

As described before, the transformation from nectar into honey means that most of the water is evaporated. Let us consider 100 mg of nectar, which consists of e.g. 85 mg of water and 15 mg of sugar (so water content of 85% and sugar content of 15%). This becomes honey with a water content of about 20%; this means 15 mg of sugar plus 3.75 mg of water, so 81.25 of the initial 85 mg of water have evaporated during this transition (i.e. 96% of the water). If the nectar has a higher sugar content, the percentage of water that evaporates is of course smaller (see Figure 15 for the relationship between this percentage and the sugar content of the nectar). Sugar contents of nectar vary between typically 10 and 50% (US-EPA white paper, p. 192). This corresponds with evaporation of 97 and 75% of the water, respectively.

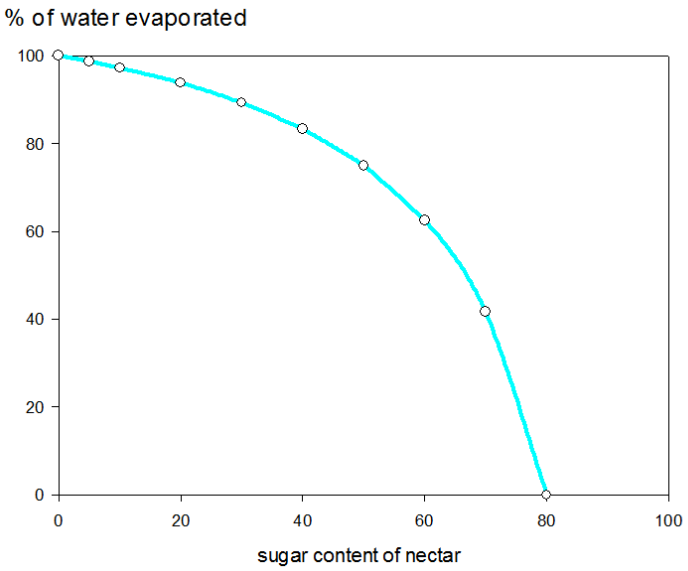

Figure 15: Percentage of water evaporated from nectar during the honey ripening process as a function of the sugar content of the nectar, assuming that ripe honey has a maximum water content of 20% (and thus a sugar content of 80%)

The evaporation of the water results from the exchange of water vapour between the surface of a shrinking nectar/honey droplet and the air in the hive (see Figure 16). Inevitably, the pesticide molecules in this droplet will undergo a similar mass transfer. Therefore, it is not defensible to ignore volatilisation of pesticide molecules from the nectar during the transition into honey. The extent of the volatilisation of the pesticide molecules depends of course on the air-water partitioning properties of the pesticide (i.e. the water solubility and the saturated vapour pressure of the pesticide).

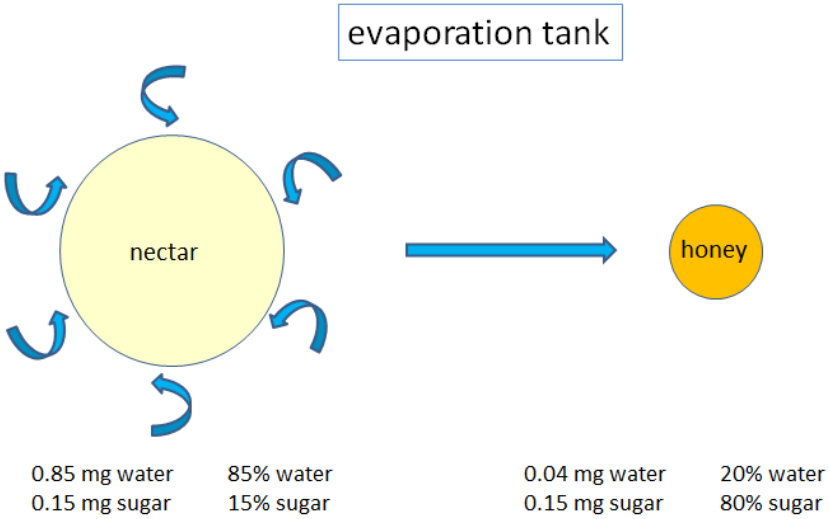

Figure 16: Schematic representation of evaporation of water during the transformation of nectar into honey. The numbers refer to a hypothetical example of 1 mg of nectar with a sugar content of 15% at the start of the transformation process

The evaporation of water and volatilisation of the pesticide molecules is modelled by considering the nectar droplet as a sphere that shrinks due to the evaporation of water (see Figure 16). It is assumed that the mass transfer rates of both the water and the pesticide are linearly proportional to the concentration difference (of the water vapour and the pesticide molecules) between the surface of the

liquid phase and the air in the hive. It is furthermore assumed that the concentrations in the gas phase at the surface of the liquid phase of both the water vapour and of the pesticide are in equilibrium with the liquid phase. This is a standard approach for mass exchange phenomena (i.e. the concept of a laminar boundary air layer; Bird et al., 1960).

The saturated water vapour concentration above a sugar solution is lower than that above pure water because the presence of the sugar molecules limits the rate of exchange of the water molecules between the liquid and gas phase; this reduction is described by Raoult's law.

It is assumed that the mass transfer coefficient for the exchange of pesticide molecules between the liquid and gas phases is based on the same thickness of the laminar boundary air layer as the mass transfer coefficient for the exchange of water vapour molecules between the liquid and gas phases. This thickness of the boundary layer can be calibrated using available measured time courses of the water content in nectar/honey systems.

For the estimation of the volume of the nectar/honey, assumptions have to be made on the density of water/sugar mixtures. It is proposed to assume that this density can be calculated simply from the density of the components of the mixture. This is justifiable as deviations from this approach are limited to percents (see measured densities of glucose solutions in combination with the density of glucose of 1.54–1.56 kg/L reported by Weast, 1974, p. C-311).

The endpoint of the calculation is the percentage of pesticide mass volatilised during the ripening of the honey. This is determined by only one pesticide property, i.e. the coefficient for distribution between liquid and gas phases (called the Henry coefficient). This coefficient can be estimated from the water solubility and the saturated vapour pressure (which are available in any pesticide dossier). The Henry coefficient is a function of the temperature because the water solubility and the saturated vapour pressure are also a function of the temperature. Default values for the activation energies of these temperature dependencies can be taken from Anonymous (2014). It is proposed to assume a temperature of the nectar droplet of 35°C during the transition from nectar to honey.

The extent of exchange between gas and liquid phase during the ripening process of honey will depend on the percentage of water that is evaporated: the more water has to evaporate, the more intensive the exchange will be. As discussed above, this percentage ranges from about 75 to 97% considering the expected range of the sugar content of the nectar. In view of this 75–97% range, it is advisable to consider the percentage volatilised to be a function of the sugar content of the nectar.

The RPU-ED modules as described in Section 8, require as input hourly values of air temperature and air humidity. It is therefore proposed to simulate the evaporation of water in the hive using these quantities as input variables. The volatilisation of pesticide can be simulated using the concept of the laminar boundary air layer as described above.

Thus, the box in the upper left corner of Figure 14 has been described and we move to the processes of honey storage and ripening. It is proposed that two processes are considered: equilibrium partitioning between the honey and the wax wall in the honey cells and first-order degradation in this honey. Hörig (2015), showed that partitioning between honey and wax is an important process: assuming equilibrium partitioning the pesticide mass in the wax exceeds the pesticide mass in the honey when the log K_{ow} (i.e. the logarithm of the octanol–water coefficient) of the substance is larger than about 0.5. The majority of the pesticides has a log K_{ow} > 0.5 (e.g. that of imidacloprid is 0.57). Equilibrium partitioning seems justifiable, as the expected residence time of the honey in the cells will usually be weeks or months. See Hörig (2015), for information on the dimensions of the cells and on the partitioning properties of both the honey and the wax. The partitioning can be based on the octanol–water partitioning coefficient of the pesticide.

We move now to the description of the concentration in the beebread. As described in the section on the consumption of nectar and food products, it is assumed that there are several classes of beebread cells of 1000 cells each, class 1 being closest to the brood and class 6 being at the farthermost distance to the brood. See this section also for the rules for emptying and filling of these cells. The pesticide concentration in each individual cell is calculated as follows:

the starting point of the calculation is the pesticide concentration in the pollen used to fill the cell and the pesticide concentration in the wax wall of the cell at the moment of filling (this concentration in the wax is the result of previous beebread storages in this cell)
it is assumed that there is equilibrium partitioning between the wax wall and the beebread (based on cell dimensions and partitioning parameters described by Hörig (2015).

first-order degradation may occur in the beebread but no degradation in the wax is assumed.
Calculations by Hörig (2015), showed that also partitioning from beebread into the wax wall is an important process (although less important than for honey cells): at a log K_{ow} of 1, about 20% of the pesticide mass is present in the wax wall at equilibrium.

As described in the section on the consumption of nectar and food products, the cells in class 1 are preferentially emptied and filled because of their proximity to the brood. Furthermore, the emptying takes place with preference for the cells with the shortest storage times. This has as a consequence that the residence time of many of the beebread in cells in this class is limited to one or two days. It is not yet certain whether it is justifiable to assume equilibrium partitioning for such short storage times. It is recommended to check this assumption by calculations with a diffusion model for a range of substance properties. An alternative approach would be to simulate the partitioning by a first-order rate equation for the exchange between the beebread and the wax wall.

The concentration in the jelly is based on the concentrations in the honey and the beebread that is taken by the nurse bees. Hörig (2015, Chapter 5) proposes to use a physiologically based toxicokinetic (PBTK) model for describing the pesticide behaviour in the nurse bees, including the pesticide concentration in the HPGs. Simulations and measurements for a few substances showed concentrations in the royal jelly from this gland that were one or two orders of magnitude lower than those in nectar fed to the nurses. According to Hörig (2015), this is caused by the limited transfer of pesticides from the intestine of the bees into the HPG. The resulting large decrease in concentration in the jelly has potentially a large influence on the simulated effects for the larvae and the queen. The model has been tested for a few substances only. Therefore, the available information on this topic should be critically examined before they are generalised for their further use in the model.

The last element of the module for the pesticide concentrations is the concentration in the larvae cells (i.e. the box at the bottom of Figure 14). This concentration is based on the concentrations in royal jelly, beebread and honey that are added into the older larvae cells by the nurse bees (see Section 9.39.3). The processes to be considered in these cells are the uptake by the larvae and the partitioning between the liquid phase in the larvae cells and the wax wall.

It can be expected that partitioning of pesticide between the liquid phase in the larva cells and the wax has a significant effect on the pesticide concentration in this liquid phase. Therefore it is proposed to simulate also the pesticide concentration in each individual larva cell (following a random pattern of cells with larvae). The partitioning between the liquid phase in the larvae cell and the wax wall of this phase has to be based on the composition of this liquid phase in terms of water, carbohydrates, lipids and proteins (see Hörig 2015 Chapter 4).

In view of the large change in the amount of liquid phase and in the type of food during the development of a larva (e.g. Haydak, 1968, reported a strong decrease of proteins and a strong increase of carbohydrates during larvae development), it is advisable to calculate this partitioning in each cell on a daily basis.

Also for the larvae cells it is not yet clear whether it is justifiable to assume equilibrium partitioning as daily concentrations are required for the effect assessment and as concentrations in the jelly entering the larvae cells may vary considerably from day to day. So also for the larvae cells it is recommended to check the equilibrium assumption by calculations with a diffusion model for a range of substance properties.

9.4. Conceptual model for pesticides consumption

The demands in sugar and protein will determine the food consumption of the bees and the food consumption will determine the pesticide intake. To estimate the pesticide intake (RI as residue intake), the pesticide concentration of the food items should also be known (see Section 9.2.3). As the pesticide concentrations are measured and reported for pollen and nectar and not for protein and sugar; the protein content of the pollen (beebread) and the sugar content of the nectar (and/or honey) should also be considered. The recommended linkage between sugar and protein demand and the pesticide residue intake is illustrated in Table 4. It is noted that this table is not comprehensive, but should be finalized once the related literature search is finalized (see more explanations below).

The sugar and protein demands of adult worker bees should be linked with the activity/activities in a given day performed by the bees. For example, foragers may perform several foraging trips to collect food from plants or water from the landscape. Therefore, the energetic demand of foragers could be linked to the flying time on that day. However, the sugar and protein demands of the queen could be linked with the number of laid eggs on that day and the demands of larvae should depend on their age. To elaborate the most important activities/drivers of the different bees/bee stages and to quantify the related sugar and protein demands, information from the known literature should be obtained. Ones the variables mentioned above are identified and quantified; the pesticide residue intake of the different bees/bee stages can be calculated using the following formula:

$$Residue\ Intake = \frac{\left(PECn * \frac{Csn}{msn}\right) + \left(PECh * \frac{Csh}{msh}\right) + \left(PECp * \frac{Cp}{mp}\right) + \ (PECj * Cj)}{1000}$$

Keys for this formula are reported in the table overleaf.

Table 4: Framework for linkage between foraging, C-HP, Pesticides and RPU-ED modules

	State variable from Foraging and Colony modules	Food consumption (mg/bee/day)			State variable from Pesticides module and RPU-ER modules Concentration of the pesticide (mg/kg), sugar concentration w/w (mg/mg) and protein concentration w/w (mg/mg)						
	Energy demand (J)	**Sugar**	**Protein**	**Royal jelly**	**Nectar**	**Sugar content of nectar**	**Honey (diluted with water)**	**Sugar content of honey**	**Beebread**	**Protein content of beebread**	**Royal jelly**
Bee stage/activity											
Nectar Forager before departing the hive	Ed daily average	Cs = Ed/En	0	0	PECn	msn	PECh	msh	NR	NR	NR
Nectar Forager returning to the hive[(a)]	Ed daily average	Cs = Ed/En	0	0	PECn	msn	PECh	msh	NR	NR	NR
Pollen Forager	Ed daily average	Cs = Ed/En	0	0	PECn	msn	PECh	msh	NR	NR	NR
Water Forager	Ed daily average	Cs = Ed/En	0	0	PECn	msn	PECh	msh	NR	NR	NR
Brood attending Nurse	Ed daily average	Cs = Ed/En	Cp	0	PECn	msn	PECh	msh	PECp	mp	NR
In-hive bees other than nurse	Ed daily average										
1-day old worker and drone larvae	NR	0	0	Cj	NR	NR	NR	NR	NR	NR	PECj
2-day old worker and drone larvae	NR	0	0	Cj	NR	NR	NR	NR	NR	NR	PECj
3-day old worker and drone larvae	NR	0	0	Cj	NR	NR	NR	NR	NR	NR	PECj
4-day old worker and drone larvae	NR	Cs	Cp	Cj	PECn	msn	PECh	msh	PECp	mp	PECj
5-day old worker and drone larvae	NR	Cs	Cp	Cj	PECn	msn	PECh	msh	PECp	mp	PECj
6-day old worker and drone larvae	NR	Cs	Cp	Cj	PECn	msn	PECh	msh	PECp	mp	PECj
7-day old drone larvae	NR	Cs	Cp	Cj	PECn	msn	PECh	msh	PECp	mp	PECj
8-day old drone larvae	NR	Cs	Cp	Cj	PECn	msn	PECh	msh	PECp	mp	PECj
9-day old drone larvae	NR	Cs	Cp	Cj	PECn	msn	PECh	msh	PECp	mp	PECj
Worker and drone pupae	NR	0	0	0	NR	NR	NR	NR	NR	NR	NR
Winter bee	Ed daily average	Cs	Cp	0	PECn	msn	PECh	msh	PECp	mp	NR
Queen		0	0	Cj	NR	NR	NR	NR	NR	NR	PECj
Drone											
Others											

(a) It is known from literature that foragers always consume 5–10% of nectar (Gary andLorenzen (1976), reported that 5–10% of nectar of the crop may be involuntarily taken into the proventriculus during foraging). It is also known that they consume increasingly nectar if they fly more than 2 km. However, the exact amounts are unknown. Exposure to residues in nectar is worst case compared to exposure to residues in honey (due to dilution and degradation). In the absence of detailed data, we suggest the following until further data become available: foragers consume 10% of nectar collected and if they fly to a patch which is further away than 2km they cover their total energy demand for the flight back from freshly collected nectar.

Keys:
Ed: energy demand
En: energetic content of sugar (e.g. sucrose: 17 J/mg)
Cs: sugar consumption, which can be Csn sugar consumption originating from nectar and/or Csh sugar consumption originating from honey
Cp: protein consumption
Cj: jelly consumption
PECn: pesticide concentration in nectar
PECh: pesticide concentration in honey
PECp: pesticide concentration in beebread
PECj: pesticide concentration in jelly
msn: sugar mass in nectar
msh: sugar mass in honey
mp: protein mass in beebread
NR: not relevant

9.5. Conceptual model for pesticides effects

9.5.1. Lethal effects

The link between the consumption of pesticide and the effects of this consumption is a crucial element (visualised by the arrows in Figure 12). Table 5 describes the elements of this link for the different exposures. The linking is based on the Ecotoxicologically Relevant Exposure Quantity (EREQ). This is a new concept and thus requires some explanation. The EFSA opinion on good modelling practice (EFSA PPR Panel, 2014, p 46) indicates that the linking between the exposure and effects is based on the Ecotoxicologically Relevant Concentration (ERC). However, Table 5 (which is based on the effect assessment of bees in the EFSA bee guidance) indicates that the concentration terminology is a too narrow concept. Therefore, it is proposed that this concept is replaced with the concept of the Ecotoxicologically Relevant Exposure Quantity (EREQ). Quantity is a general term based on the terminology used in the Système International (SI) system of units (Bureau International des Poids et Mesures, 1998); examples of quantities are time, length, mass, volume, concentration etc. This EREQ is the interface between the different exposure and effect assessments as indicated in Table 5.

Table 5: Overview of the different effects considered together with the ecotoxicological endpoints on which these are based and corresponding exposure quantities that determine these effects

Effect	Category of bee	Exposure route	Time aspect	Endpoint from lab test–input for the model	Ecotoxicologically Relevant Exposure Quantity (EREQ)	Unit
Mortality	Adult worker bee	Contact	Acute	LD_{50} and slope	Mass of pesticide attached to one forager bee resulting from a single application	µg
Mortality	Adult worker bee	Oral	Acute	LD_{50} and slope	Mass of pesticide consumed by a forager during a single flight	µg
Mortality	Adult worker bee	Oral	Acute	LD_{50} and slope	Daily mass of pesticide consumed by one nurse bee daily mass of pesticide consumed by one forager bee	µg
Mortality	Adult worker bee	Oral	Chronic (10 day)	LD_{50} and slope (also LC_{50})	10-days average of daily mass of pesticide consumed by one nurse bee 10-days average of daily mass of pesticide consumed by one forager bee	µg
Mortality	Larva	Oral + contact	Chronic (3–4 day)	NOEL LD_{50} and slope is not required by EU, but by US, therefore sometimes available sometimes not (also LC_{50})	Cumulative mass of pesticide consumed by a larva or maximum of daily mass consumed by larva or maximum of daily concentrations in the liquid phase in larva cell	µg or µg/kg
Sub-lethal on HPG	Adult worker bee (only nurse bee)	Oral	Chronic (10 day)	NOEL (slope unlikely be available)	10-days average of daily mass of pesticide consumed by one nurse bee	µg

Thus, for all categories of bees shown in Table 5, dose–response relationships are needed showing how each category is affected by the pesticide. We assume that dose–relationship is the same for all these types of bees presently based on young bees. In this context, the dose is the EREQ as defined in Table 5. The response is the percentage or probability of mortality (see Figure 17 for an example). For non-toxic substances only a NOEL will be available (e.g. NOEL > 100 µg per bee); thus, the model should also provide a NOEL as an input option.

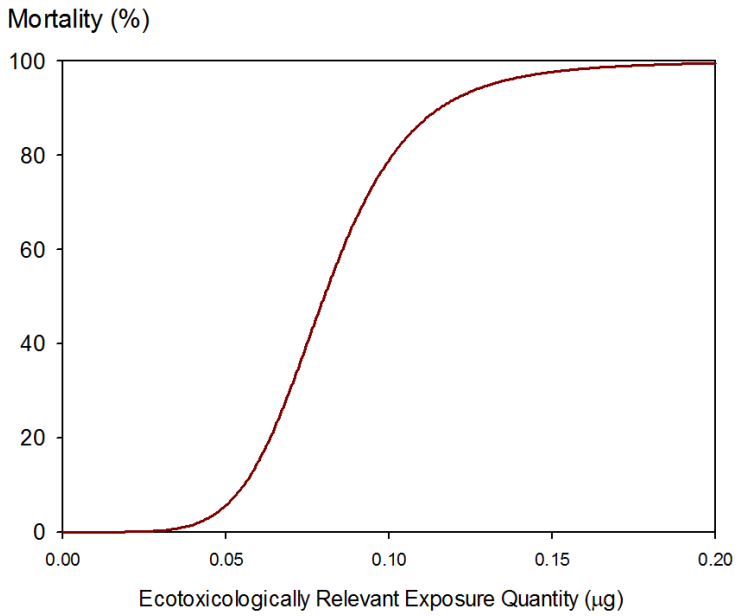

Figure 17: Example of a dose–response curve showing how the mortality varies with the Ecotoxicologically Relevant Exposure Quantity (the unit used for this quantity is arbitrary)

As shown in Figure 12, the effects on larvae are caused both by consumption and contact exposure because they consume the liquid phase around them. Table 5 indicates that the effect studies with larvae implicitly include both contact and oral effects. This topic will require further attention during the model development.

As described in Section 7 and Table 4, the consumption needs of nurses and foragers differ between summer and winter. Moreover, there are indications that also the sensitivity of these bee categories to pesticides differs between summer and winter. Therefore, the model should contain the option to use different acute and chronic dose–response curves (i.e. for both lethal and sublethal) for summer and winter.

9.5.2. Sublethal effects

The following endpoints should be considered in the model:

- Reproductive performance of queens, measured through queen fecundity and replacement.
- HPG development as an indicator of brood care.
- Effects on homing ability, measured through the number of non-returning bees to the hive (i.e. foragers considered dead) or through the number of bees delayed upon their return to the hive.

Reproductive performance of queens

This should be implemented in the model *via* a reduction factor (to be applied to the egg-laying rate) that depends on the pesticide mass consumed by the queen (it has been shown that specific pesticides (e.g. bifenthrin and deltamethrin) reduce the fecundity of the queen and the ability of the colony to raise a new one (Dai et al., 2010).

Hypopharyngeal glands development

Effects on the HPG development may result in effects on the brood care or in earlier foraging. The effects on brood care might be described by a reduction of the number of brood cells that a single nurse can take care of or by a reduction of the number of nurses that can take care of brood. However, there are still considerable uncertainties with respect to the quantitative linking between effects on HPG development and brood care effects. Therefore it is proposed not to include effects of HPG development in the model at this stage but instead to generate model output that indicates (as a function of time) when the NOEL for the HPG is exceeded (a traffic-light type of output). Once sufficient data become available to implement dose–response relationships between HPG development and brood care then this should be implemented in a future model. However, it has been shown that exposure to pesticides could cause a 15% decrease in HPG size, which would equal to a shift in foraging earlier by at least 6 days and consequently to a reduction of life span by 6 days (Rueppell et al., 2007; Smodiš Škerl and Gregorc, 2010; Khoury et al., 2011; Hatjina et al., 2013).

Homing ability

The consumption of nectar during foraging is described in Table 4. As indicated in Figure 12, the effects of consumption of pesticide during flying have to be evaluated separately, following the dose–response curve for the acute toxicity (as described in Table 5). For sublethal effects, the dose–response curve may be different to the one for acute effects. For an assessment of effects from sublethal exposure on homing flight ability, it would be necessary to implement this in the model by increasing the flight time back to the hive proportionally to the effect observed in specific homing flight studies. Homing flight studies would need to be designed in such a way that the time to return to the hive is measured for individual bees (see Bortolotti et al., 2003; Henry et al., 2012, 2014).

9.5.3. How to deal with mixture toxicity

The following parts of this section are from the scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (EFSA PPR Panel, 2012).

Concentration addition (CA)

This approach is used where chemicals have the same site of action (simple similar joint action) but do not affect the biological activity of each other (no interaction). For this method the endpoint must be the same for each chemical.

Total Toxicity = (Ca/Ta + (Cb/Tb) +.....+ (Cn/Tn) Concentration addition (CA)

Where C = concentration (or dose)

T = toxicity

In a recent review for the EC (Kortenkamp et al., 2009), the use of the concentration addition model was proposed as the concept of mixture toxicity that is most relevant for hazard characterisation and ultimately can be integrated into the legislative process for risk management purposes. The use of the concentration addition has also been discussed by Verbruggen and van den Brink (2010). There are two reasons that make the use of this model concept attractive for policy makers. First, the model concept is generally more conservative than the concept of response addition. Nevertheless, the magnitude of the differences at low levels of exposure between the two models is usually small and, hence, the outcome will not be overly conservative. A second reason for the use of concentration addition is that the model concept can make use of existing data such as a NOEC (No Observed Effects Concentration), EC_{10} or EC_{50} (effective dose that produces a level of effects of 10% and 50%, respectively) by applying the concept of toxic units (TUs).

The concept of TUs has been recently reviewed by the three non-food committees of the EC [the Scientific Committee on Health and Environmental Risks (SCHER), the Scientific Committee on Emerging and Newly Identified Health Risks (SCENHIR) and the Scientific Committee on Consumer Safety (SCCS)]. These committees defined TUs as 'the ratio between the concentration of a mixture component and its toxicological acute (e.g. LC_{50}) or chronic (e.g. long-term NOEC) endpoint'. In addition, the toxic unit of a mixture (TUm) has been defined as the sum of TUs of each individual chemical of that mixture. The committees also noted that the TUs concept only refers to a specific

organism representative of a group of organisms ecologically or taxonomically relevant for the ecosystem (e.g. algae, daphnids and fish for the freshwater ecosystem) but not to the ecosystem as a whole (SCHER/SCENIHR/SCCS, 2011).

The following equation can be used for deriving a surrogate EDx, ECx, NOEC or NOEL value for a mixture of active substances with known toxicity assuming dose additivity:

$$1/EC_x\,(\text{mix}) \text{ or } 1/NOEC\,(\text{mix}) = \left(\sum_i \frac{X(a.s._i)}{EC_x\_or\_NOEC(a.s._i)} \right)$$

Where:

X(a.s.i) = fraction of active substance [i] in the mixture (please note that the sum Σ X(a.s.i) must be 1) EC$x$or NOEC(a.s.i) = toxicity value for active substance [i] (for the same endpoint).

Response addition (Ra)

This approach is used where chemicals have different sites of action (independent joint action) but do not affect the biological activity of each other (no interaction). Here each component of the mixture acts on a different physiological or biological system but contributes to a common response. This requires biological response (BR) expressed as % toxic effect for the assessed concentration from dose–response curve for each constituent.

Total toxicity = BR1 + BR2 + ... +BRn Response addition (Ra), see figure below.

The disadvantage of this method is that it requires dose–response data for all of the mixture constituents and species being assessed, but that is what we are aiming for in our model so this would be an approach to follow.

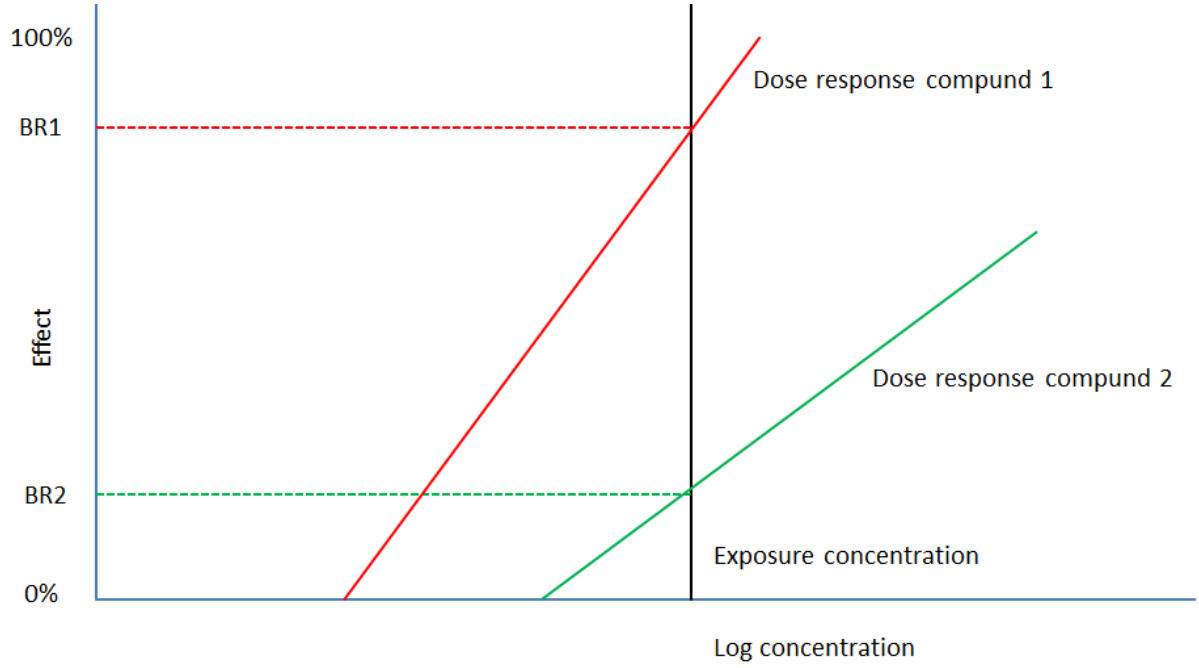

Figure 18: Biological response (BR) expressed as % toxic effect for the assessed concentration from dose–response curve for two compounds

9.6. State variables for the Pesticides module

All state variables required for the Pesticides module are summarised in Tables 6 and 7.

Table 6: State variables required as input for the Pesticides module

Variable	Unit	Time resolution
Mass attached to forager bees resulting from single pesticide application	µg	h
Flow rate of nectar entering the hive (mass of nectar per time period)	g/d	h
Flow rate of pollen entering the hive (mass of pollen per time period)	g/d	h
Sugar content of nectar entering the hive (mass of sugar divided by mass of water)	-	h
Flow rate of water entering the hive (mass of water per time period)	g/d	h
Pesticide concentration in nectar entering the hive (mass of pesticide per mass of nectar for a certain time period)	mg/kg	h
Pesticide concentration in pollen entering the hive (mass of pesticide per mass of pollen for a certain time period)	mg/kg	h
Pesticide concentration in water entering the hive (mass of pesticide per mass of water for a certain time period)	mg/kg	h

Table 7: State variables described by the Pesticides module

Variable	Unit	Time resolution
Pesticide concentration in nectar/honey in evaporation tank	mg/kg	1 day
Pesticide concentration in honey	mg/kg	1 day
Pesticide concentration in wax in nectar evaporation tank	mg/kg	1 day
Pesticide concentration in wax in honey	mg/kg	1 day
Pesticide concentration in beebread preparation tank	mg/kg	1 day
Pesticide concentration in beebread in individual beebread cells	mg/kg	1 day
Pesticide concentration in wax in individual beebread cells	mg/kg	1 day
Pesticide concentration in jelly provided by nurses	mg/kg	1 day
Pesticide concentration in liquid phase of individual larva cells	mg/kg	1 day
Pesticide concentration in wax of individual larva cells	mg/kg	1 day
Daily mass of pesticide consumed by a larva	mg	1 day
Daily mass of pesticide consumed by a nurse bee	mg	1 day
Daily mass of pesticide consumed by a forager bee	mg	1 day
Daily mass of pesticide consumed by the queen	mg	1 day
TWA 10-days of pesticide mass consumed by a nurse bee	mg	10 days
TWA 10-days of pesticide mass consumed by a forager bee	mg	10 days
TWA 4-days of pesticide mass consumed by a larva	mg	4 days
Daily consumption of jelly by a larva	mg	1 day
Daily consumption of beebread by a larva	mg	1 day
Daily consumption of nectar by a nurse	mg	1 day
Daily consumption of honey by a nurse	mg	1 day
Daily consumption of beebread by a nurse	mg	1 day
Daily consumption of nectar by a forager	mg	1 day
Daily consumption of jelly by the queen	mg	1 day
Daily consumption of nectar by a drone	mg	1 day
Mass of nectar in evaporation tank	mg	1 day
Mass of honey	mg	1 day
Number of beebread cells filled with beebread	-	1 day
Is individual beebread cell filled, yes or no ?	-	1 day
Time elapsed since filling of individual beebread cell	d	1 day

10. Biological Agents module

10.1. Effects of the biological agents on the colony

Based on the recommendations made by the HEALTHY-B WG and the PPR Panel on the BEEHAVE model, the following biological agents were considered for the model implementation:

- *Varroa destructor,*
- Deformed Wing Virus,
- Acute Bee Paralysis Virus,
- *Nosema ceranae.*

For each of these biological agents, their effects on the Colony, Foraging and in-Hive Products modules were summarised below in diagrams (see Figures 19 and 20) and further described to specify which trait(s), variable(s) and life stage(s) are impacted (see Tables 8 and 9 and references in Appendix B).

10.1.1. Varroa destructor and associated viruses: DWV and ABPV

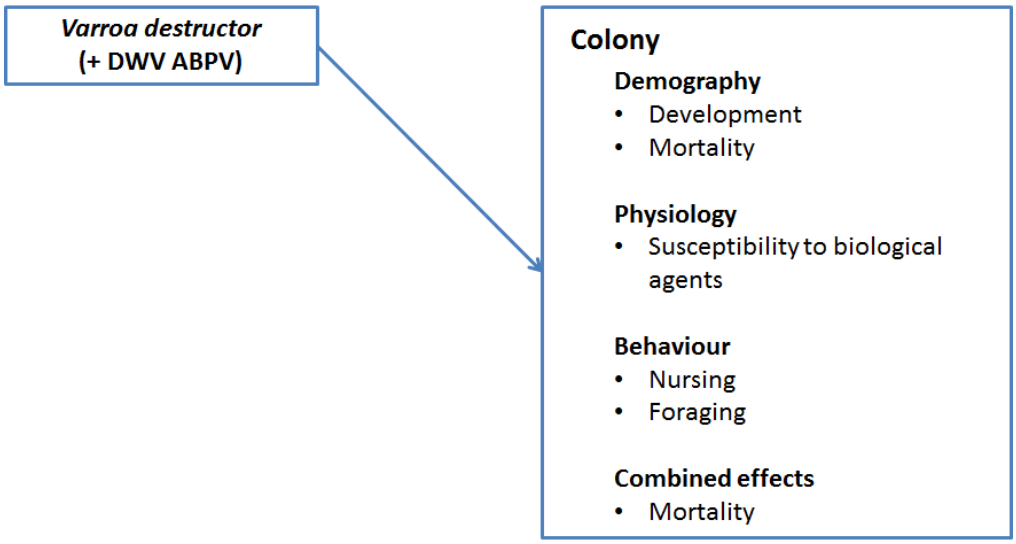

Figure 19: Schematic figure showing the effects of *V. destructor* and its associated viruses on Colony module

Table 8: Detailed description showing the effects of *V. destructor* (and related viruses) on each trait, variable and life stage within the Colony module

Trait	Variable	Life stage	Effect
Demography	Development	Egg	More than 75% of the eggs will be infected with at least one virus.
Demography	Development	Larva	Reduced weight especially when > 1 mites in the cell (with DWV and ABPV viruses)
Demography	Development	Pupa	Reduced weight of about 7-10% especially when > 1 mites in the cell The reduction in adult weight can be 30% if the immature bee is infested by more than 3 mites
Demography	Development	Nurse	Become foragers earlier; increased development rate; reduced life span
Demography	Development	In-hive bee (non-nurse)	Become foragers earlier; increased development rate. Depending on prevalence (both during pupae development and on adult bee) and deformation level, impossibility to become foragers (impaired wings)
Demography	Mortality	Pupa	100% of ABPV infected pupae are dead and 20% of DWV-infected pupae are dead
Demography	Mortality	Nurse	Increased mortality; deformed wings bees die within 48 hours. Bees infected with ABPV die within 8 days
Demography	Mortality	In-hive bee (non-nurse)	Increased mortality
Demography	Mortality	Forager	Reduced homing ability; decreased capability of non-associated learning, prolonged absences from the colony and a lower rate of return to the colony (reduced ability to navigate). This will lead to increased mortality
Demography	Mortality	Winter bee	DWV is strongly associated with winter colony mortality
Demography	Mortality - combined effects	Larva	Synergistic effect on colony mortality if in association with tracheal mites and American foulbrood
Physiology	Susceptibility to biological agents	Larva	*V. destructor* is increasing the susceptibility to chalkbrood and stonebrood infections, American foulbrood, and tracheal mite
Physiology	Susceptibility to biological agents	Pupa	*V. destructor* mites can induce immunosuppression in parasitized pupae and activate covert virus infections
Physiology	Susceptibility to biological agents	In-hive bee (non-nurse)	ABPV facilitate a co-infection with other viruses
Physiology	Susceptibility to pesticides	In-hive bee	*Varroa* infestations can be higher in imidacloprid treated colonies
Behaviour	Foraging	Forager	Impossibility to foragers to perform their activity (deformed wings), depending on prevalence (both during pupae development and on adult bee) and deformation level

10.1.2. Nosema ceranae

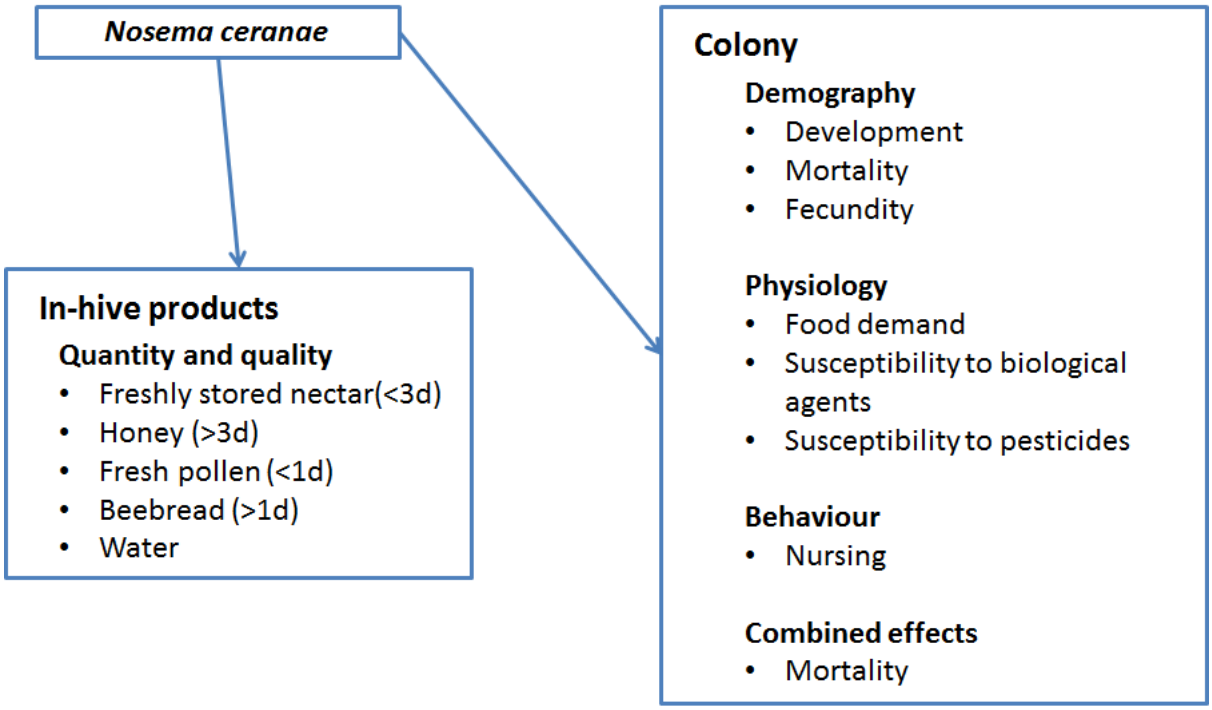

Figure 20: Schematic figure showing the effects of *N. ceranae* on Colony and in-Hive Products modules

Table 9: Detailed description showing the effects of *N. ceranae* on each trait, variable and life stage within the Colony module

Trait	Variable	Life stage	Effect
Demography	Development	Adult bee	Shorter life-spans (live 9 days less)
Demography	Development	Forager	Infected bees are nearly twice as likely to engage in precocious foraging Progressive and irreversible degeneration of the gut epithelium that can lead to disorders of the digestive function
Demography	Mortality	Nurse	Increased mortality
Demography	Mortality	Forager	Increased mortality: mortality may be dependent on the infectious dose, higher temperatures (e.g. South Europe) Homing success is significantly reduced in *N. ceranae* infected (65.8%) versus healthy foragers (92.5%).
Demography	Mortality - combined effects	Forager	Combination of *V. destructor* and *N. ceranae* could augment the risk of colony death. Synergistic interaction with imidacloprid. Significant increase in honeybee mortality occurred when young workers were infected by *N. ceranae* and then chronically exposed to sublethal doses of the insecticides thiacloprid (neonicotinoid) or fipronil (phenylpyrazol)
Physiology	Food demand	Drone, nurse	Alters the energy demand by elevated hunger; decreases hemolymph sugar level
Physiology	Susceptibility to biological agents/pesticides	Drone, nurse	Suppresses the bee immune response/sensitize the honeybees to chemical stressors
Physiology	Susceptibility to pesticides	Forager	*N. ceranae* induces susceptibility to organophosphate pesticides and neonicotinoids
Physiology	Susceptibility to biological agents	Winter bee	Synergistic effects between *N. apis* and several honeybee viruses such as filamentous virus (FV), bee virus Y (BVY) and black queen cell virus (BQCV). Both *N. Apis* and BQCV were found in colonies that collapsed during winter
Behaviour	Nursing	Nurse	Malformation of the hypopharyngeal glands and fat body, reduced quantity of royal jelly produced

Table 10: Detailed description showing the effects of *N. ceranae* on quality and/or quantity of in-hive products.

Target	Quality/quantity	Effect
Stored fresh nectar (stored nectar < 3 days), honey (stored nectar > 3 days), fresh pollen (stored pollen < 1 day)	Quality	Contamination with spores
	Quantity	Reduced quantity due to population decline
Beebread (stored pollen > 1 day), water	Quality	Contamination with spores
	Quantity	N.A.

11. Beekeeping Management Practices (BMP) module

11.1. Selection of relevant BMP for the model

The MUST-B WG considered the 17 BMP described by the Healthy-B WG and assessed their relevance for the model development, in particular their potential effects on the different modules (i.e. core module comprising the Foraging and C-HP modules, the Biological Agents and Pesticides modules). Finally, the two BMP 'beekeeper category' and 'beekeeper experience' were grouped together ('beekeeper category and experience'). As a result, a final selection of six BMP was made (Table 11).

Table 11: Selection of the BMP to be considered for the model development

List of BMP from HEALTHY-B	Effects on Pesticides module	Effects on Biological Agents module	Effects on core module	Selection of BMP for the model
'Introduction of a queen bee'	No	Yes	Yes	No[(a)]
'Change in the number of workers'	**No**	**Yes**	**Yes**	**Yes**
'Production type of the colony'	No	No	No	No
'Migration activity'	No	No	Yes	No[(b)]
'Chemical control'	**Yes**	**Yes**	**Yes**	**Yes**
'Replacement of combs with brood'	**Yes**	**Yes**	**Yes**	**Yes**
'Replacement of combs with feed sources'	**Yes**	**Yes**	**Yes**	**Yes**
'Supplementary feeding'	**No**	**No**	**Yes**	**Yes**
'Beekeeper category and experience'	**No**	**Yes**	**Yes**	**Yes**
'Location of the apiary'	No	No	No	No
'Proximity of colonies belonging to other apiaries'	No	Yes	No	No[(c)]

(a): the replacement of the queen may have an influence on the Biological Agents and core modules, but all other demographic, physiological and behavioural traits will also be affected. Thus, the recommendation is that the queen should not be replaced during the risk assessment, and if natural replacement (supersedure or swarming) occurs, the assessment should be over.

(b): the change of location of the colony for production may have an influence on the core module. However, during the risk assessment, the colony should remain at the same location.

(c): the proximity of other colonies belonging to other apiaries may have an influence on the Biological Agents module (spread of diseases among colonies). However, as a first step of development, the model considers a single colony (without interactions) and therefore this BMP is not included but it could be developed in the future when more data is collected.

Effects of each of the selected BMP are further described below and in Appendix C.

11.2. Effects of beekeeping management practices on the model

11.2.1. Change in the number of workers

According to HEALTHY-B, 'Change in the number of workers' is the introduction and removal of bees at the colony level 'typically in-hive bees'.

This practice does not involve movement of frames, but only the introduction or removal of bees (typically in-hive) at colony level. If a colony is judged to be 'weak', the beekeeper may decide to add worker bees, either in the form of package bees (bees with no frame) or simply by shaking one or more frames of bees from a typically stronger colony to a typically weaker one. If a colony is judged 'strong', the beekeeper may decide to remove bees, either as a measure to prevent swarming, or for production of package bees for commercial purposes. Thus, the quantity of adult bees of both a donor and a receiver colony is modified. Typically, a donor colony is stronger than a receiver colony. Receiver and donor colonies are subject to different consequences.

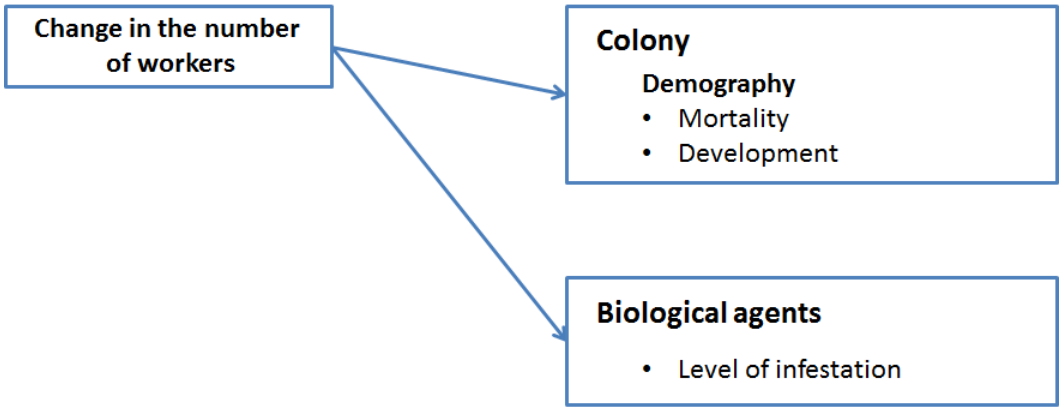

Figure 21: Schematic figure showing the effects of 'change in the number of workers' on Colony and Biological Agents modules

Table 12: Modules, variables and stages affected by 'change in the number of workers'

Module	Variable	Stage	Effect
C-HP demography	Mortality	Immature stage	Survival of the brood may be impaired if the ratio adult bees/brood is too low (< 0.5)
Biological Agents	Level of infection/infestation	Direct effect on adult, indirect effects on immature stage	The receiver colony will have positive effects against pest and disease from the increased adult/brood ratio if the introduced bees are not infected and/or infested. However, if the introduced bees are infected/infested (and if the level of infestation is higher than the level in the receiver colony), this will result in an increased level for the receiver colony.

11.2.2. Chemical control

According to HEALTHY-B, 'Application of chemical control methods' is the chemical control method used, including active agents, dosages and timing, and respective target.

Description of practice: in Europe, only acaricide treatments are allowed (antibiotics against bacterial and fungal diseases are permitted only in exceptional situations in a few countries). Thus, the effects of this BMP here refer only to authorised treatments for the control of *V. destructor*, including the ones authorised for organic beekeeping (essential oils, organic acids). As stated at the beginning of this paragraph, we consider that the treatment is applied according to Good Beekeeping Practices (GBP), thus following the label indications and in the appropriate environmental conditions.

However, it is important to note that *V. destructor*, depending on regions and strains, may show resistance to some chemical controls (even approved ones) (Lodesani and Costa, 2005; Sammataro et al., 2005).

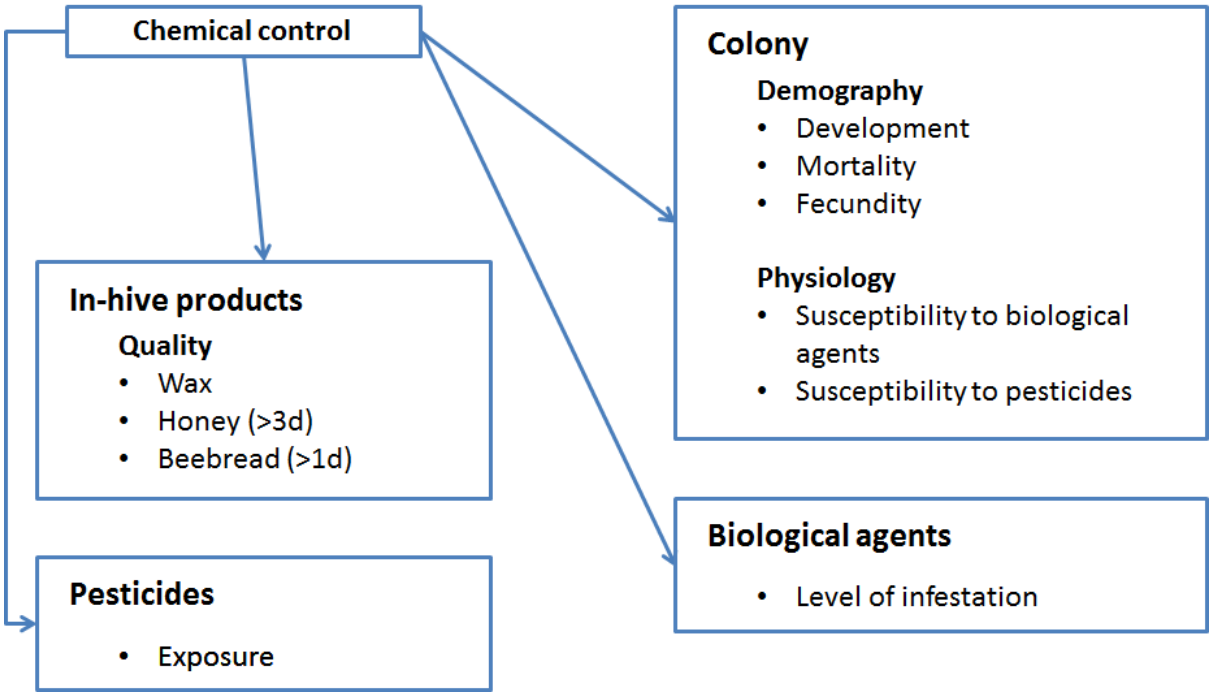

Figure 22: Schematic figure showing the effects of 'chemical control' on Colony, in-Hive Products, Biological Agents and Pesticides modules

Table 13: Modules, variables and stages affected by 'chemical control'

Module	Variable	Stage	Effect
C-HP demography	Development	Immature stage	Delayed development in worker bees is observed in bees reared in contaminated combs containing high levels of pesticides
C-HP demography	Mortality	In-hive bee (specifically, bees raised in laboratory conditions, up to 19 days of age)	Adult longevity is reduced in bees exposed to pesticide residues in contaminated brood comb during development.
C-HP demography	Mortality	Larva	Coumaphos and tau-fluvalinate show chronic oral toxicity to honeybee larvae, alone and in combinations. The larval mortality is over than two-fold compared to non-exposed larvae
C-HP demography	Mortality	Queen	Increased mortality in queen
C-HP demography	Fecundity	Queen	Queens reared in acaricide-laden beeswax (coumaphos and fluvalinate) show significantly lower sperm counts and viability, and higher mating frequency, compared to queens reared in miticide-free beeswax
C-HP physiology	Susceptibility to biological agents and pesticides	All bees	Treatments with coumaphos and fluvalinate cause significant changes in genes involved in detoxification, behavioural maturation, immunity, and nutrition (study on

Module	Variable	Stage	Effect
			genome-wide gene expression patterns of honeybee workers). Following treatments with coumaphos, alteration of metabolic responses (detoxification gene expression pathways, components of the immune system responsible for cellular response and developmental genes) were observed, which could potentially interfere with the health of individual honeybees and entire colonies
C-HP In-Hive Products	Residues in bee matrices such as wax, beebread and honey	All bees (immature and adult)	Chemicals are often found as residues in various bee matrices such as wax, beebread and honey. Most acaricides are lipophilic, thus the matrix with highest risk of residues is beeswax. If the concentration of acaricide residues in beeswax is high, the molecules may then pass into the stored honey. Contamination may also be indirect, i.e. from old wax to new wax. Treatment with amitraz can lead to residues in honey. Oxalic acid and thymol may residue in honey if not applied according to GMP
Biological Agents	Level of infestation	Mostly direct effect on adult bees – with the exception of formic acid which can reach mites in the brood. Direct effect on brood (less mites can enter the brood, less adult bees transmitting viruses)	Chemical substances are used to reduce biological agents (e.g. *Varroa*) prevalence. If applied correctly (and if there is no resistance phenomena developed in mites), efficacy of the authorised products reaches 99%. Successful control of *Varroa* limits replication and transmission of DWV and ABPV
Pesticides	Residues in bees and bee matrices	Depends on the type of treatment but generally impacts all bees	Treatment implies exposure of the bees to a pesticide inside the colony

11.2.3. Replacement of combs with brood

According to HEALTHY-B, 'replacement of combs with brood' is the introduction or removal of combs with brood from the colony.

A comb containing brood sources is added or removed from the colony. If combs are added, the study-colony is defined 'receiver', otherwise 'donor'. Adult bees and feed sources could be present on the comb. If a colony is weak, the beekeeper may decide to add combs with brood, either with or without adult bees. If a colony is strong, the beekeeper may decide to remove brood combs, either as a measure to prevent swarming, or to use for strengthening weak colonies, or for the production of nukes (small colonies with or without queen) for commercial purposes. Thus, the quantity of brood of both a donor and a receiver colony is modified. If the comb contains adult bees, the quantity of adult bees could be modified too. Typically, a donor colony is stronger than a receiver colony. Receiver and donor colonies are subject to different consequences. The quantity of brood and adult bees in the managed combs should be defined to better define the impact of this practice on the colony. Since we assume GBP, we exclude the situation in which the brood is added to a colony that is not able to appropriately rear it (i.e. lack of adult bees).

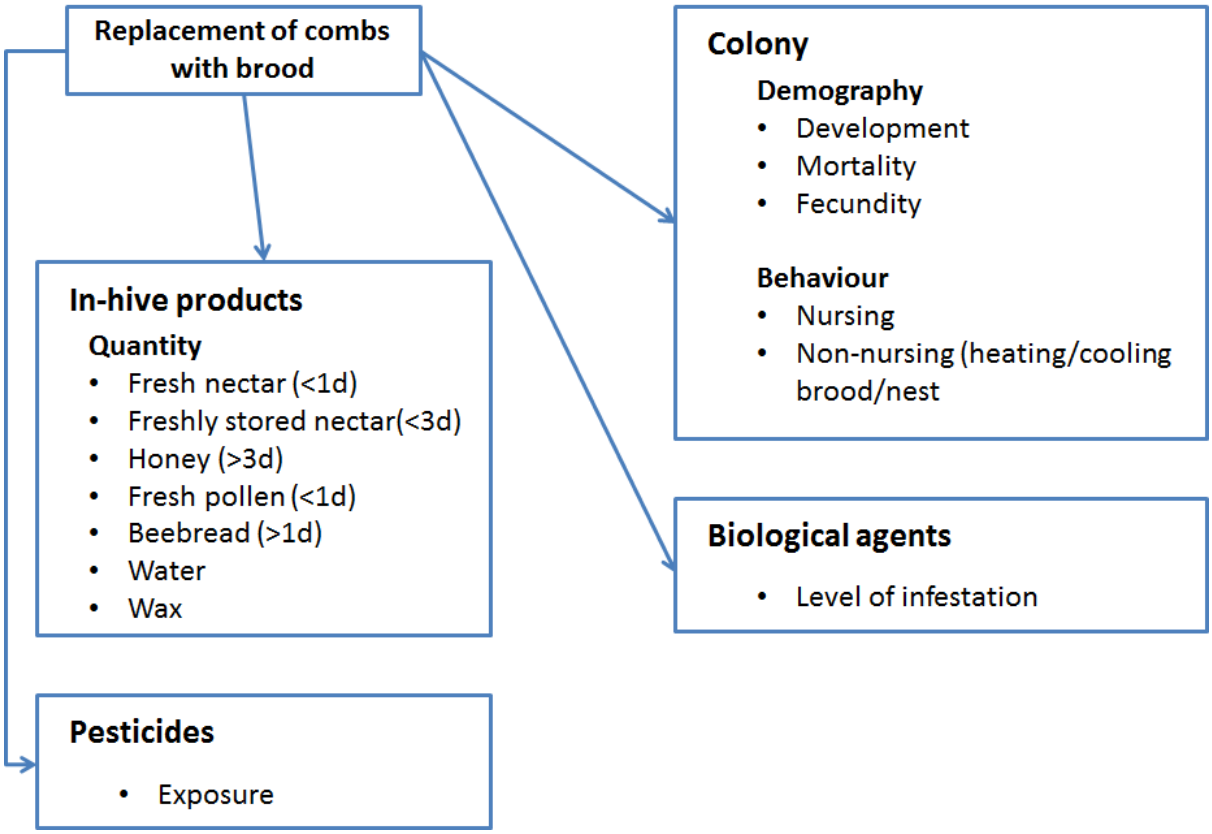

Figure 23: Schematic figure showing the effects of 'replacement of combs with brood' on Colony, in-Hive Products, Biological Agents and Pesticides modules

Table 14: Modules, variables and stages affected by 'replacement of combs with brood' for the receiver colony

Receiver colony			
Module	**Variable**	**Stage**	**Effect**
C-HP Demography	Number of immature and adult bees	Immature	Increased population of immatures and adult (if present) bees in the receiver colony. Decreased immature and adult bees (if present) in the donor colony
C-HP Behaviour	Oviposition rate	Queen	The egg-laying rate depends on demography and food availability.
C-HP Behaviour	Nursing	Nurse	Brood rearing requires nurse bees time and energy
C-HP Behaviour	Heating/cooling brood	In-hive adult	An increase in the number of bees thermoregulating and nursing.
C-HP Behaviour	Worker task	Nurse, non-nurse, forager	Depending on the new adult/brood rate (i.e. need of nursing to rear brood/need of foraging to collect food), possible task modification of bees

Table 15: Modules, variables and stages affected by 'replacement of combs with brood' for the donor colony

Donor colony			
Module	**Variable**	**Stage**	**Effect**
C-HP Demography	Worker task	Nurse, non-nurse, forager	Depending on the new adult/brood rate, possible task modification of bees that used to be nurses and now can do other tasks: e.g. increase of foraging.
C-HP In-Hive Products	Quantity of stored food introduced	All bees	There will be an increase in the food stored in the receiver colony and decreased food stored in the donor colony.
Biological Agents	Level of infestation	Immature and adult bee	Brood may contain infectious agents and pests (e.g. Varroa mites, *Nosema* spp., etc.) that may spread within the new colony. Bacterial diseases infect immature stages and thus may be present in the brood. The varroa mite reproduces in brood and thus may also be present, together with its associated viruses. Other viruses may be present (sacbrood virus-SBV, CBPV). If the donor colony was infected by *Nosema* spp., faeces containing infectious spores may be present on the combs
Pesticides	Contact exposure	Brood and adult bee	Possible exposure if the comb contain contaminated food, either in the brood cells or stored.

11.2.4. Replacement of combs with feed sources

According to HEALTHY-B, 'replacement of combs with feed sources' is the introduction or removal of food combs containing pollen and/or honey stores.

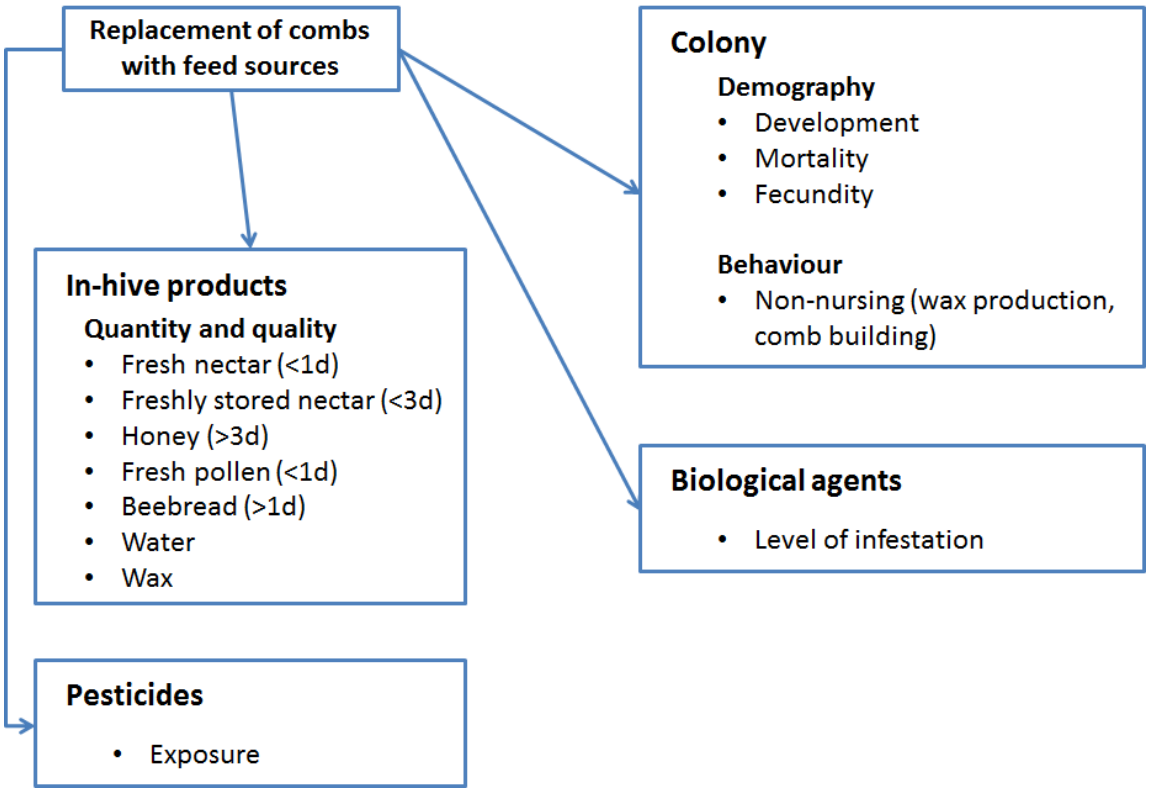

Figure 24: Schematic figure showing the effects of 'replacement of combs with feed sources' on Colony, in-Hive Products, Biological Agents and Pesticides modules

Table 16: Modules, variables and stages affected by 'replacement of combs with feed sources', in a donor or receiver colony

Module	Variable	Stage	Effect
C-HP Demography	Number of immatures and adult bees	Depends on which stage is introduced	If adult bees are moved together with the comb, increased adult bees quantity in the receiver colony and decreased adult bees quantity in the donor colony
C-HP Behaviour	Oviposition rate	Queen	The egg laying rate depends on demography and food available.
C-HP Behaviour	Comb building	Non-nurse	The timing of comb building is influenced by two variables that need to occur simultaneously: nectar availability/influx and stored food quantity/comb fullness (> 80%). The conditions required to initiate comb building are different from those required to maintain the building. To continue comb building, the colony needs to continue experiencing a high nectar influx.
C-HP In-Hive Products	Quantity of food sources introduced	All bee stages	Quality and quantity of the food introduced alters the nutritional status of the colony (i.e. in-hive products), increasing (receiver colony) and decreasing (donor colony) the quantity of in-hive products.
Pesticides	Residues in bees and bee matrices	Immature and adult bees (depending on the matrices that are contaminated)	The introduction of food combs carries risks related to the possible contamination by pesticides (i.e. both acaricides and agrochemicals
Biological Agents	Level of infestation	Immature and adult bees (depending on biological agent)	The introduction of food combs carries risks related to the possible contamination by biological agents

11.2.5. Supplementary feeding

'Supplementary feeding' refers to the practice of providing feed (i.e. sugars, proteins and amino acids, lipids, micronutrients, probiotics, water) to the colony to improve its nutritional status, especially in periods of necessity (i.e. dearth). Supplementary feeding is typically provided through the insertion of combs with food produced by bees (i.e. honey, beebread), liquid (i.e. syrups) or solid (i.e. candies) artificial supplements (i.e. mostly made of sugars). This practice is typically more common and intense in terms of quantity and time of provision among professional beekeepers.

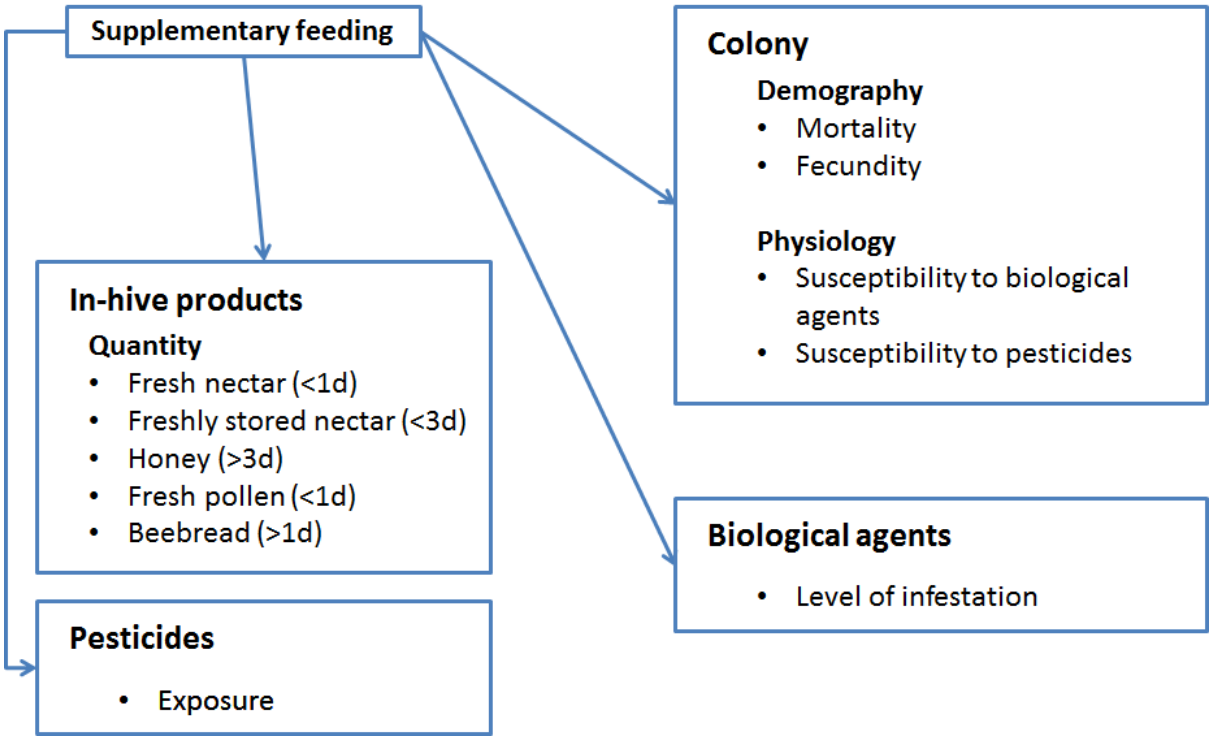

Figure 25: Schematic figure showing the effects of 'supplementary feeding' on Colony, in-Hive Products, Biological Agents and Pesticides modules

Table 17: Modules, variables and stages affected by 'supplementary feeding'

Module	Variable	Stage	Effect
C-HP Demography	Fecundity	Queens	Increased egg laying if liquid feed is provided in spring / summer
C-HP Demography	Longevity	All bees (immatures and adults)	If the supplementary feeding is provided in period of dearth, it can increase the longevity of adult bees, reduce the mortality of immatures, which may otherwise even be cannibalised
C-HP Physiology	Susceptibility to biological agents and pesticides	All bees (immatures and adults)	DWV virus concentrations increased as bees aged and were highest in those fed sugar syrup and lowest in bees fed pollen
C-HP In-Hive Products	Quantity of food stored	All bees (immatures and adults). Depending of food type (e.g. pollen/beebread or sugar/honey), different stages of bees could be involved	If supplementary pollen is provided it may be stored as beebread. Honey and sugars may also be stored

Module	Variable	Stage	Effect
Biological Agents	Exposure	All bees (immatures and adults). Depending of food type (e.g. pollen/beebread or sugar/honey) and biological agents, different stages of bees could be involved	Feed may contain spores (i.e. bacteria / fungi) or viruses which may thus be transmitted to the colony which is being fed. This depends on the kind of feed (e.g. honey or pollen)
Pesticides	Exposure	All bees (immatures and adults). Depending of food type (e.g. pollen/beebread or sugar/honey), different stages of bees could be involved	If the supplementary feed is contaminated, it will expose bees to the contaminant directly or, if stored, through the contaminated stored food

11.2.6. Beekeeper category and experience

According to HEALTHY-B, 'Beekeeper category' (hobbyist versus professional) and 'experience' is the level of expertise of the person/body in charge of data collection for field survey is linked to skills gained through practice and training (e.g. years of experience, training level, number of colonies managed).

'Beekeeper category and experience' is a critical parameter in field surveys conducted by beekeepers, but not for the model to be used in RA as it is assumed that the colony would be managed according to good beekeeping practices (GBP). However, the definition of what is a 'GBP' varies across EU (depending on abiotic and biotic factors) and cultures (traditions). Therefore, further work is required before a proper definition is provided.

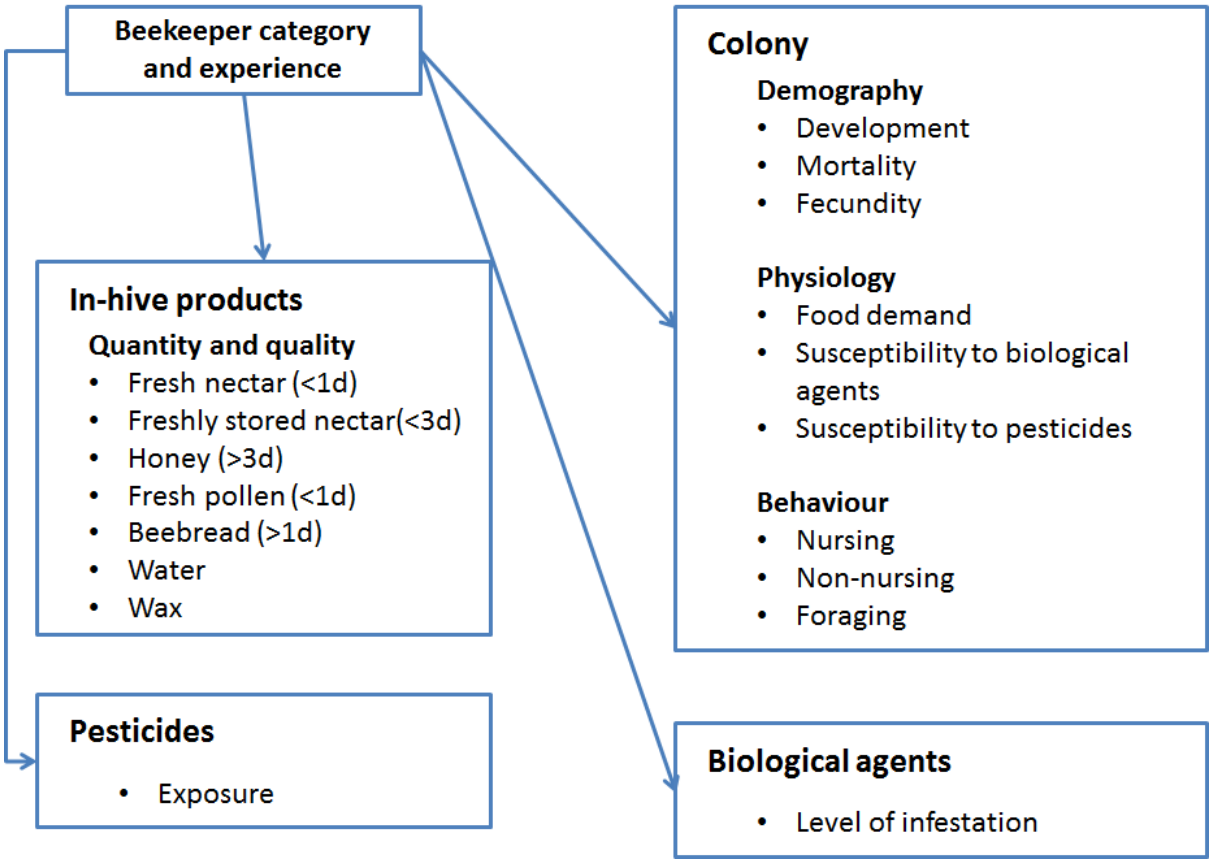

Figure 26: Schematic figure showing the effects of 'beekeeper category and experience' on Colony, in-Hive Products, Biological Agents and Pesticides modules

12. Conclusions

The development of the conceptual model presented in this technical report for the risk assessment of pesticides on honeybee colony under different scenarios of resource providing unit and environmental drivers, biological agents and beekeeping management practices was a challenging task. This development was based on expert knowledge and data retrieved from the literature.

Data are available to define the structure of the modules and to describe the processes characterizing the dynamics of the colony, the in-hive products, the foraging activities and the exposure to pesticides. Important data gaps and incomplete knowledge were found affecting the fine description and parameterization of some biological or environmental processes. This introduces some complexity and uncertainty in the development of the different modules of the model. Information was either not available or not possible to find within the timeline of this mandate. In some cases, the working group made some suggestions (framework to calculate and extrapolate values); in other cases, the working group highlighted areas that need to be further investigated, for example:

- Pesticides module:
    - Acute and chronic dose–response curves (i.e. for both lethal and sublethal) for summer and winter bees.
    - Contact and oral exposures in larvae *via* the liquid phase of their food which is also in contact with the surface of the brood comb (wax).
    - Sublethal effects on queens (e.g. *via* impairment of pheromone production), nurses (e.g. *via* impairment of brood care) and foragers (e.g. *via* impairment of homing behaviour).
- Resource Providing Unit and Environmental Drivers module:
    - Predicting non-crop resource availability, including the production of pollen and nectar from non-crop plants in margins.
- Beekeeping Management Practices module:
    - Effects of the beekeeping management practices 'production type of the colony', other than 'honey production', on honeybee colony health with quantitative data.
    - The working group considered optimal 'Good Beekeeping Practices' in the model (for risk assessment purpose), but a detailed definition of how to assess 'beekeeper category and experience' for inclusion in the model is still required.

For the design of the model that is proposed in this report, the working group capitalised on the existing knowledge and EFSA's recommendations (EFSA PPR Panel, 2015). The working group made substantial progress by developing a model that is broader in scope and capacity to expand in the future. For this work, the working group underwent a systemic and innovative analysis of the dynamics of a honeybee colony and its interactions with the environment and stressors.

13. Recommendations

- The conceptual model presented in this report shall form the basis for the development of a model to be used to assess the impact of pesticides on honeybee colony health in complex landscapes in the presence of multiple stressors. The ultimate goal is to design a model for further, including regulatory, use by risk assessors and risk managers.
- This report provides the specifications needed for the outsourcing of the model development.
    - The information (data and references) presented in this report was collected by expert knowledge, but needs to be thoroughly checked and completed with other sources of information (e.g. research, databases, scientific literature, pesticides post-registration monitoring studies, etc.). Literature reviews shall be conducted with justified criteria and methodology and the selected papers shall be critically reviewed.
    - The computer model should be written in a computer language that accommodates the computational burden required by the spatial and temporal resolutions envisioned (1 m, 1 h to 1 day). It should also allow for future expansion, as outlined below, including the integration of new evidence as it becomes available.
    - To calibrate and evaluate the model, data collected from a number of study sites in Europe will be outsourced as a separate project to the model development. Requirements for field data collection are currently being developed and will complement the work of HEALTHY-B on validated methods and tools for measuring indicators of honeybee colony health in field surveys. Throughout the project, it will be critical that there is ongoing interaction between these two project teams, to maximise project synergies.
- A data model shall be elaborated by the EFSA Bee Task Force and validated by the MUST-B working group in order to collect data in an appropriate and standardised format. The data shall be stored on a platform managed by EFSA and accessible by the contractors (to store and use data).
- The progress of the model development shall be regularly monitored and supported by an EFSA Steering Committee. This Committee shall be made of members of the MUST-B working group and the EFSA Bee Task Force.
- Finally, MUST-B has the objective to develop a holistic approach to risk assessment. However, to avoid over-parameterisation, the model only considers a subset of stressors and factors. Epidemiological and other studies should be conducted, to determine the relative importance of multiple stressors and factors involved in colony losses in order to define opportunities for further development of the model.
- The conceptual model developed by the working group is a first step. New opportunities for further development were identified by the working group, in particular in the following areas:
    - The potential for exposure to multiple chemicals.
    - The addition of new biological agents.
    - The inclusion of multiple colonies in a complex landscape.
    - The inclusion of dynamic processes when considering multiple stressors such as biological agents.

The model should be developed in a way that it is easily expanded once new evidence data become available. A version control would be required for the use of the model for regulatory purposes, and potential implementations should be clearly described (i.e. what would be updated and by when).

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Acronyms

A	Application rate
ABPV	Acute Bee Paralysis Virus
AHAW Panel	EFSA Panel on Animal Health and Welfare
ALMASS	Animal, Landscape and Man Simulation System
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
ABPV	Acute Bee Paralysis Virus
BBCH	Bundesanstalt, Bundessortenamt und CHemische Industrie
BK	Beekeeper
BMP	Beekeeping management practices
BQCV	Black Queen Cell Virus
BR	Biological Response
BVY	Bee Virus Y
C	Concentration (or dose)
Co	Food products consumed by bees
CA	Concentration addition
CBPV	Chronic bee paralysis virus
C-HP	Colony and in-hive products
Cj	Jelly consumption
COLOSS	Cost Action Project on 'Prevention of Honeybee colony losses'
Cp	Protein consumption
Cs	Sugar consumption
DEB	Dynamic Energy Budget
DDT	Dichlorodiphenyltrichloroethane
DT_{50}	Dissipation half-life
DWV	Deformed Wing Virus
E	Estimated linear slope constant
EC	European Commission
EC*x*(a.s.*i*)	Effect concentration where x % effect was observed/calculated for active substance [i]
EC_{10}	Effect concentration where 10 % effect was observed/calculated
EC_{50}	Effect concentration where 50 % effect was observed/calculated
ED	Environmental drivers
Ed	Energy demand
En	Energetic content
EFSA	European Food Safety Authority
Eqn	Equation

ERC	Ecotoxicologically Relevant Concentration
EREQ	Ecotoxicologically Relevant Exposure Quantity
EU	European Union
EURL	European Union Reference Laboratory
Fe	Cumulative exposure probability
fdep	Deposition factor
FV	Filamentous Virus
GBP	Good Beekeeping practices
GMP	Good Modelling Practice
Ha	Food products handled by bees
HPG	Hypopharyngeal gland
JRC	Joint Research Centre
K	Rate constant
K_{ow}	Logarithm of the octanol–water coefficient
I	Infected
IAPV	Israeli Acute Paralysis
KBV	Kashmir Bee Virus
LC_{50}	Median Lethal concentration
LD_{50}	Median Lethal dose
LOD	Level of detection
LOQ	Level of quantification
PG	Protection Goal
m	Mass of the chemical deposited/bee
msn	Sugar mass in nectar
msh	Sugar mass in honey
mp	Protein mass in beebread
MUST-B	EU efforts towards the development of a holistic approach for the risk assessment on MUltiple STressors in Bees
NOEC	No Observed Effect Concentration
NOEC(a.s.*i*)	No Observed Effect Concentration for active substance [*i*]
NOEL	No Observed Effect Level
NR	Not relevant
P/N	Parasite per host
PBTK	Physiologically Based ToxicoKinetic
PEC	Predicted Environmental Concentration
PECn	Predicted Environmental Concentration of pesticides in nectar
PECh	Predicted Environmental Concentration of pesticides in honey
PECp	Predicted Environmental Concentration of pesticides in beebread
PECj	Predicted Environmental Concentration of pesticides in jelly

PPR Panel	EFSA Panel on Plant Protection and their Residues
R	Removed
RA	Risk Assessment
Ra	Response addition
RH	Relative humidity
RI	Residue Intake
RPU	Resource providing unit
RUD	Residue Unit Dose
S	Susceptible
SBPV	Slow Bee Paralysis Virus
SC	Scientific Committee
SCER	Scientific Committee and Emerging Risk
SCHER	Scientific Committee on Health and Environmental Risks
SCCS	Scientific Committee on Consumer Safety
SI	Système International
SPU	Service Providing Unit
T	Toxicity
TF	Task Force
TU	Toxic Unit
TUm	Toxic Unit of a mixture
USA	United States of America
US-EPA	United States Environmental Protection Agency
US-EPA-OPP	United States Environmental Protection Agency Office of Pesticide Programs
WG	Working Group
X(a.s.*i*)	Fraction of active substance [*i*] in the mixture

Glossary

Assessment endpoint	An explicit expression of the environmental value to be protected, operationally defined as an ecological entity and its attributes (Suter et al., 1993).
Attribute	Only a few high level statements describing the key criteria of bee health in a holistic sense at the colony level. Attributes are multidimensional and hence cannot be measured directly.
Calibration	Adjusting one or more input parameters to improve the match between model output and experimental data.
Computer model	A model that describes the mathematical model in code that can be executed by a computer, this does not include the actual values of the input parameters.
Conceptual model	A model in which the elements are described explicitly and in which their mutual dependencies are described; conceptual models are usually described in words or *via* a diagram.
Deterministic model	A mathematical or computer model in which all parameters can have one unique value only and in which one parameter set results in one unique output.
Distributions of scenarios	A number of scenarios to be created which reasonably characterise the range of driving forces for the environmental fate mechanism being studied; driving forces are in this context the primary variables controlling the environmental fate mechanism.
Factor	A set of abiotic or biotic components associated with external drivers
Indicator	A set of abiotic or biotic components associated with colony attributes and colony outputs
Mathematical model	A model that describes the conceptual model in terms of mathematical equations.
Model	A simplified representation of a part of reality that contains mutually dependent elements.
Patch	A relatively homogeneous area that differs from its surroundings (Forman, 1995). A patch is the basic unit of the landscape that changes and fluctuates; It has a definite shape and spatial configuration, and can be described compositionally by internal variables such as number of trees, number of tree species, height of trees, or other similar measurements.
Probabilistic model	A mathematical or computer model which accounts for variability in one or more input parameters and expresses outputs as probability density functions; a probabilistic model is often just a deterministic model run many times.
Range of validity of the model	A part of reality to which the validation of a model applies. The range of validity of the model covers all honeybee colonies in the agricultural areas in the EU where the pesticides are applied.
Scenario	A representative combination of crop, soil, climate and agronomic parameters to be used in modelling; representative means in this context that the selected scenarios should represent physical sites known to exist, i.e. the combination of crop, soil, climate and agronomic conditions should be realistic.
Sensitivity analysis	The analysis of the degree to which the model result is affected by changes in input parameters; often done by examining the % change

	in one output caused by the % change in an input parameter; the purpose is to obtain a better understanding of the behaviour of the model.
Software package	The computer code (both source and executables) that is provided to users; so the package includes all files on the diskette(s) which will usually include also one or more scenario's and standard data sets for checking.
Stochastic model	A mathematical or computer model in which some or all parameters are handled explicitly, as stochastic variables in the governing equations of the model, and which expresses outputs as probability sensity functions.
Stressor	Any physical, chemical, or biological entity that can induce an adverse response.
Uncertainty analysis	The analysis of the degree to which the model result is affected by the uncertainty input parameters; the purpose of uncertainty analysis is to examine the effects of lack of precise knowledge of input parameters caused e.g. by natural variation or variation resulting from measurement or analytical techniques.
Validation status	The extent to which the model has successfully gone through a validation process within a specified range of validity (e.g. the validation status of the model is poor for the whole EU, but it is reasonably good for countries A and B).
Validation model	A model which has gone successfully through a validation process for a specified rang of validity; this implies that the number of data sets considered is sufficient for the intended use of the model.
Validation process	A comparison of model output with data independently derived from experiments observations of the environment; this implies that none of the input parameters is obtained *via* calibration; note that this definition does not specify any correspondence between model output and measured data.
Verification	An examination of the numerical technique in the computer model to ascertain that it truly represents the mathematical model and that there are no inherent numerical problems with obtaining a solution; this implies also a check on errors in the code (programming bugs).
Version control	The measures taken by the institute that delivers the software package to ensure that the specified number of the version identifies the package uniquely.

Part of the above definitions have been based ASTM Designation E 978 - 84 entitled 'Standout practice for evaluating environmental fate models of Chemicals' (p. 582–587 in 1990 Annual book of ASTM standards, Vol. 11.04, Section 11, Water and environmental technology).

Appendix A - Dynamic approach for future development on biological agents

Biological agents are important stressors for honeybee colonies and need to be incorporated in the model.

We suggest distinguishing the following three groups of stressors within the most known biological agents:

- Micro-parasites: e.g. bacteria (*Melissococcus plutonius*, *Paenibacillus larvae*, *Nosema apis* and *N. ceranae*), Viruses (Deformed Wing Virus (DWV), Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Slow Bee Paralysis Virus (SBPV), Israeli Acute Paralysis Virus (IAPV), Acute Bee Paralysis Virus (ABPV), Kashmir Bee Virus (KBV)), fungi (*Ascosphaera apis*; *Aspergillus spp.*) and protozoa (*Crithidia mellificae)*.
- Macro-parasites: e.g. mites (*Varroa destructor*; *Acarapis woodi*), insects (*Aethina tumida*, *Galleria mellonella*, *Achroea grisella, Senotainia tricuspis*, *Acherontia atropos*).
- Predators: wasps (*Vespa velutina*) and other species (bears, birds, ants, spiders, etc.). It is known that the dynamics of these groups of stressors are governed by different sets of ecological rules.

As outlined in Section 5.4, opportunities for model development are envisaged including the introduction of process-based dynamic descriptions of biological agents, to account for the dynamics of the agent population. With this modification, dose–response curves (Figures 27 and 28) will be dynamic through time and will be numerical input to the model and will be obtained from laboratory, field and literature studies and through real-time data collection.

A dose–response curve is an X–Y graph relating the dose of a stressor to the effect on the honeybee colony. Dose–response curves are generally or sigmoidal (log[dose]) (Verhulst, 1838, 1845) and monophasic and can be fit to a classic Hill equation (Hill, 1910). Exponential (r parameter) or beta-Poisson (alpha and beta parameter) dose–response are possible too. Statistical analysis of dose–response curves may be performed by regression methods such as the probit/logit models, or other methods, such as the Spearman–Karber method (Hamilton et al., 1977). Empirical models based on nonlinear regression are usually preferred over the use of some transformation of the data that linearizes the dose–response relationship (Bates and Watts, 1988). A generalized model for multiphasic cases has also been suggested (Di Veroli et al., 2015). See figures below for different dose–response curves (Fazil, 2005).

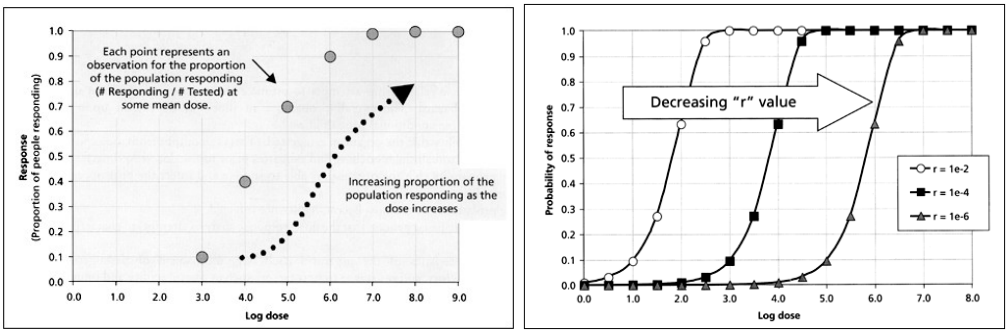

Figure 27: sigmoidal–exponential (Fazil, 2005)

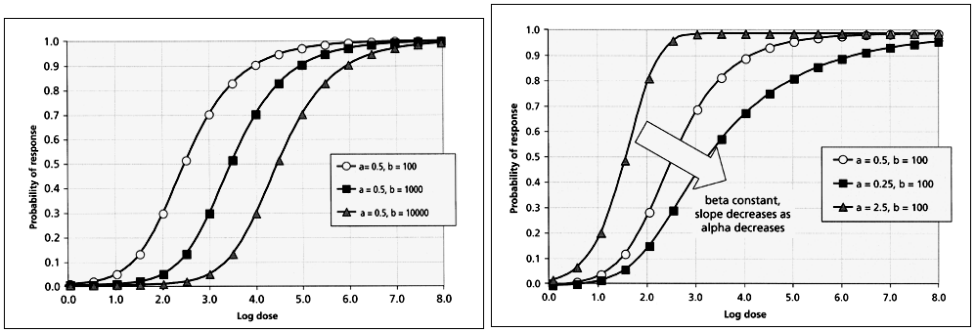

Figure 28: 2 beta-poissons (Fazil, 2005)

Modelling for Macro-parasites

According to Anderson and May (1978), three specific categories of population processes are shown to be of particular significance in stabilizing the dynamical behaviour of host-parasite interactions and enhancing the regulatory role of the parasite. These categories are:

- overdispersion of parasite numbers per individual host, following a negative binomial distribution (Figure 29). If you know the mean number of parasites per host (P/N) and k (the aggregation parameter of the negative binomial distribution), one can figure out the theoretical proportion of hosts with n parasites. If you use the pn(t) equation, you end up with a closed system;
- non-linear functional relationships between parasite burden per host and host death rate (Figures 30 and 31; Anderson and May, 1978);
- density-dependent constraints on parasite population growth within individual hosts. Provided the host population's intrinsic growth rate is positive, the parasites are capable of regulating the growth of the host population only under certain conditions.

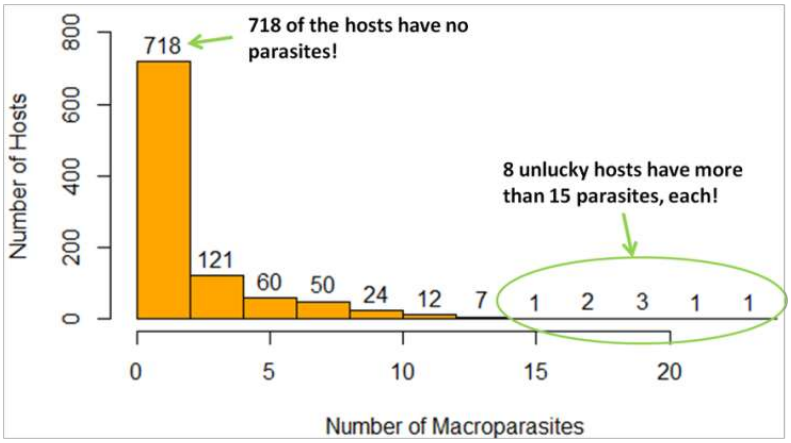

Figure 29: Parasite Aggregation in hosts. The negative binomial distribution

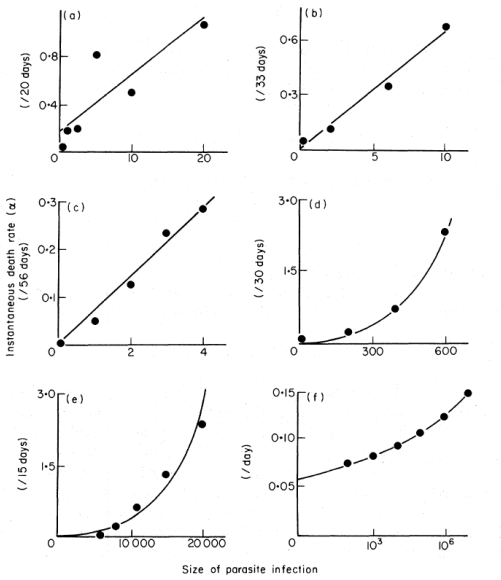

Figure 30: Some examples of functional (dose–response) relationships between parasite-induced host mortality rate (α) and parasite burden per host (i) (Anderson and May, 1978)

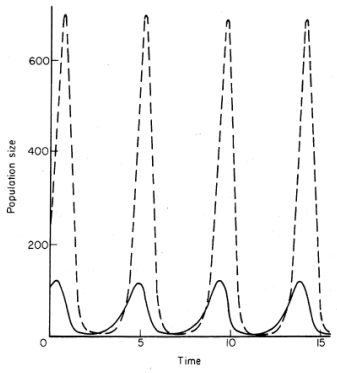

Figure 31: Resulting population dynamics for hosts and parasites (Anderson and May, 1978)

Finally, we know that infection rates might vary among hosts. If parasites/agents are not uniformly distributed in the environment – if they have a gamma distribution among patches,for instance – and then host contact with parasites within a patch is random (Poisson), one obtains a Poisson–Gamma distribution which is similar to the negative binomial distribution (May, 1978). Heterogeneity in agent distribution in the environment can cause variation in host infection rates.

Furthermore, there may also be variation in infection rates due to variation in host susceptibility. For instance, when the host's immune response is related to normally-distributed host body condition, you can theoretically get aggregation of parasites among hosts (Morrill and Forbes, 2012).

Epidemic SIR modelling for micro-parasites

An epidemic model is a simplified means of describing the transmission of communicable disease through individuals. When dealing with large populations, as in the case of a honeybee colony (adults and/or juveniles as necessary), deterministic or compartmental mathematical models can be used. The dynamics of an epidemic may be much faster than the dynamics of birth and death (colony demography), therefore, birth and death are sometimes omitted in simple epidemic models.

In the deterministic epidemic model, individuals in the population are assigned to different subgroups or compartments, each representing a specific stage of the epidemic. Letters such as Susceptible (S), Infected (I), and Removed (R) are often used to represent different stages/compartments. The SIR model without vital dynamics/demography (Kermack and McKendrick, 1927) can be expressed by

only 3 compartments (Table 18) and 3 differential equations for a fixed population (Figure 32). Extensions are possible to include demographics (death/birth: population modelling).

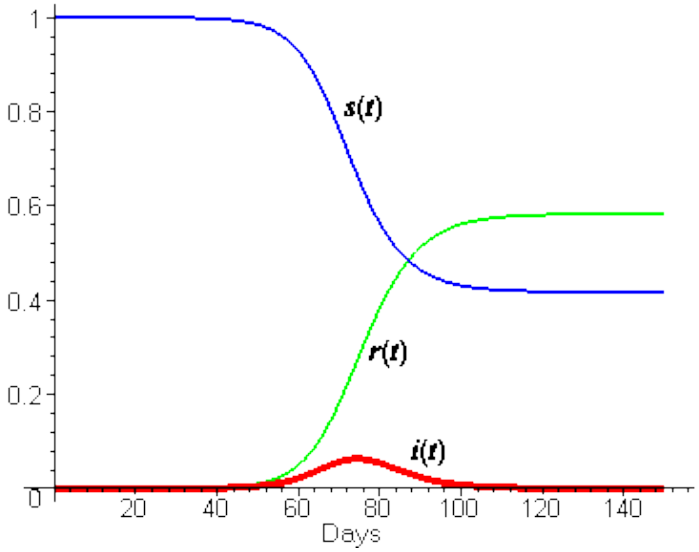

Figure 32: Example of fluxes in the three population compartments (SIR) in a fixed population (honeybees: adults and/or juveniles as necessary) for a given micro-parasite. http://www.maa.org/press/periodicals/loci/joma/the-sir-model-for-spread-of-disease-the-differential-equation-model

Table 18: Three bee life stages/compartments at time = t

S = S(t)	The absolute number of susceptible individuals that are not yet infected
I = I(t)	The absolute number of infected individuals, that are capable of spreading infection to other susceptibles
R = R(t)	The absolute number of removed (recovered or dead) individuals, not able to be infected again or to transmit infection to other susceptibles
s(t) = S(t) / N,	The susceptible fraction of the population (%),
i(t) = I(t) / N,	The infected fraction of the population (%)
r(t) = R(t) / N,	The removed fraction of the population (%)
N = S(t) + I(t) R(t) 1 = s(t) + i(t) + r(t)	The total population = total size of the honeybee colony

Table 19: SIR model flow in a fixed/closed population: S -> I -> R (without births and natural deaths):

$\frac{dS}{dt} = -\frac{\beta SI}{N}$	Susceptible equation: β = rate of contact or infection of a given micro-parasite
$\frac{dI}{dt} = -\frac{\beta SI}{N} - \gamma I$	Infected equation: β = rate of contact or infection of individuals; γ = rate of removal (recovery or death rate)
$\frac{dR}{dt} = \gamma I$	Removed equation: γ = rate of removal (recovery or death rate)
$R_0 = \frac{\beta}{\gamma}$	R0 = the basic reproduction number/ratio. This ratio is derived as the expected number of new/secondary infections from a single infected individual (I) in a population where all subjects are susceptible (S). The dynamics of the infectious class (I) depends on this ratio.
R_0, β and γ	These are micro-parasite specific values (determined in the laboratory or field)

Existing dynamic modelling applications for honeybees and important biological stressors

There are many published mathematical models available for simulating honeybee colony dynamics with(out) biological agents: see the many modelling references in Becher et al., (2013) and some output examples in the figures. The tender applicant will have to consider and review all of these references and more recent ones up to present in detail. The most influential models are listed in Table 20.

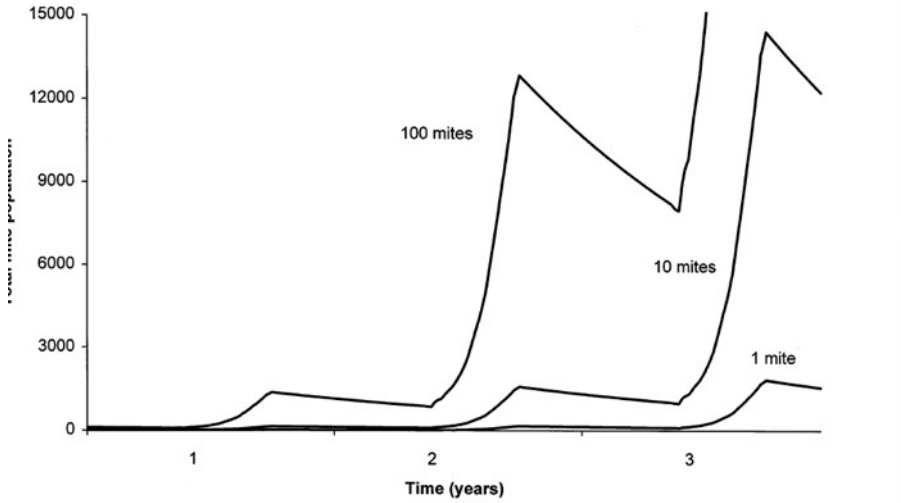

Figure 33: Predicted mite population development over a 3-year period, when starting with different (1, 10 or 100) numbers of mites (Martin, 1998)

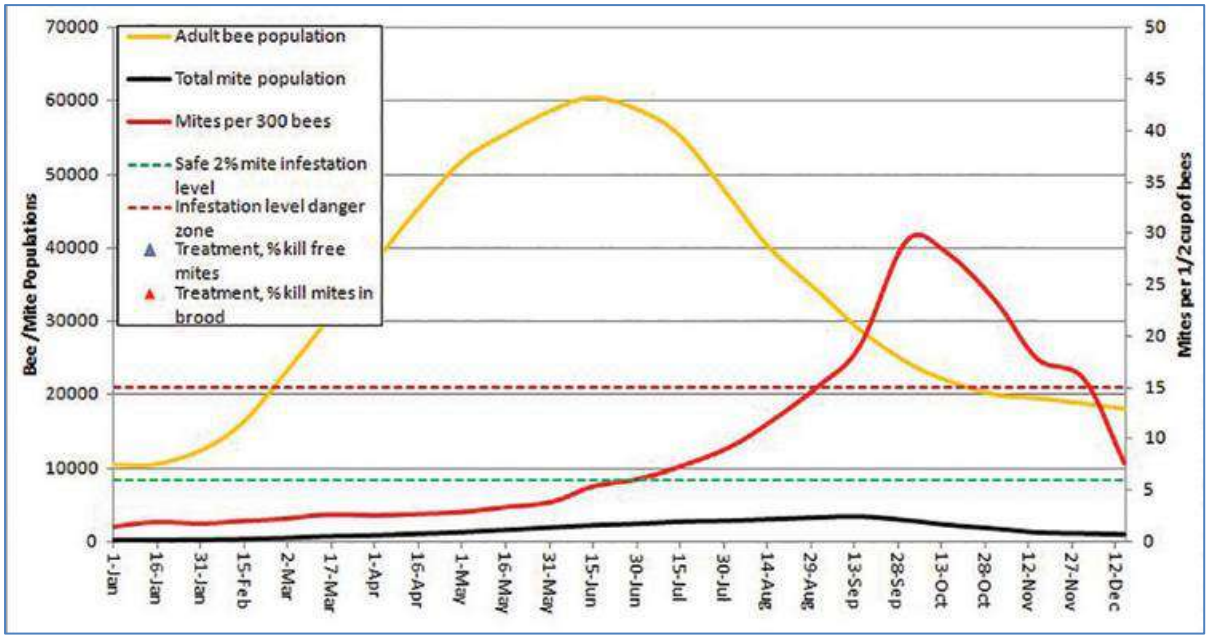

Figure 34: Dynamics of *Varroa* alone and with bees (Oliver, 2015)

Table 20: Overview of dynamic modelling examples and papers relevant to honey bees and their associated biological agents

Type of model	Literature examples
MODELING IN GENERAL	Pontryagin et al. (1962); Van den Driessche and Watmough (2002);Grimm et al. (2006, 2010); Dornberger et al. (2012); Evans et al. (2012); Grimm and Railsback, 2012; Betti et al. (2014); Eberl et al. (2014)
COLONY DYNAMICS	Simple: Matis and Kiffe (2002);Mistro et al. (2005); Al-Khafaji et al. (2009); Complex: Omholt (1986); DeGrandi-Hoffman et al. (1989); Makela et al. (1993); Thompson et al. (2005, 2007); Schmickl and Crailsheim (2007); Khoury (2009); Khoury et al.(2011, 2013); Becher et al. (2014); Russel et al.(2013); Torres et al. (2015)
COLONY + *VARROA* (+/- TREATMENT)	Omholt and Crailsheim, 1991; Fries et al. (1994); Boot et al. (1994, 1995); Martin (1998); Calis et al. (1999a,b); Wilkinson and Smith (2002); Matis and Kiffe (2002); Al Ghamdi and Hoopingarner (2004); DeGrandi-Hoffman and Curry (2005a,b); Chen et al.(2006); Vetharaniam and Barlow (2006); Okosun (2013); DeGrandi-Hoffman et al. (2014); Santos et al. (2015)
COLONY + (*VARROA* +) VIRUSES (ABPV/DWV/CBPV)	Martin (2001); Sumpter and Martin (2004); Chen et al. (2006); Eberl et al. (2010); Martin et al. (2010); Di Prisco et al. (2011); Francis et al. (2013); Ratti et al. (2013, 2015); Betti et al. (2014); Moore et al. (2015)
NOSEMA (*CERANAE*|*APIS*)	Higes et al. (2008a,b, 2009); Alaux et al. (2010); Gisder et al. (2010); Botías et al. (2013); NYMU (2013); Charbonneau et al. (2016); Lecocq et al. (2016)
COLONY + INSECTICIDES	Thompson, 2003; Thompson et al. (2005, 2007); Alaux et al. (2010); Khoury et al. (2011); Cresswell and Thompson (2012); Henry et al. (2012)
COMPLEX LANDSCAPE	Exposure: Bromenshenk et al. (1991); Lonsdorf et al., (2009); Osborne (2012); Foragers:Dukas and Edelstein-Keshet (1998)
MULTIPLE COLONIES	Calis et al. (1999b); Mistro et al. (2005); Vetharaniam and Barlow (2006); Kribs-Zaleta and Mitchell (2014)
FORAGING + DECISIONS + TASKS	Camazine and Sneyd (1991); Seeley et al. (1991); de Vries and Biesmeijer (1998, 2002); Sumpter and Pratt (2003); Higginson and Gilbert (2004); Schmickl and Crailsheim (2004); Dornhaus et al. (2006); Beekman et al. (2007); Al-Khafaji et al. (2009); Becher et al. (2010); Johnson and Nieh (2010)
LABOUR DIVISION IN SOCIAL INSECT COLONIES (Foraging-For-Work models)	Seeley et al. (1991); Tofts and Franks (1992); Franks and Tofts (1994); Svendova-Franks and Franks (1995); Huang and Robinson (1996, 1999); Robson and Beshers (1997); Beshers and Fewell (2001); Beshers et al. (2001); Leoncini et al. (2004); Schmickl and Crailsheim (2007)

Spatial modelling for biological stressors

Finally, we know that infection/infestation rates may vary among the environment. If parasites/agents are not uniformly distributed in the environment, perhaps showing a gamma distribution among patches, then host contact with parasites within a patch is random (Poisson). One would finally get a Poisson–Gamma distribution, which is for practical reasons the same as a negative binomial distribution (May, 1978). Heterogeneity in agent distribution in the environment can cause variation in bee category and colony infestation/infection/predation rates.

Potential spatial modelling techniques for biological stressors may be found in recent pollination models (expert opinion Healthy-B):

- expert opinion based 'invest' model: (Zulian et al., 2013) www.openness-project.eu,
- species distribution models with 'Maxent' (Polce et al., 2013, 2014),
- current work Joint Research Centre (JRC): interactions pollination availability EU crops and changes in policy/climate drivers.

Appendix B - Varroa destructor, its associated viruses (DWV and ABPV) and Nosema spp.

Varroa destructor

Haplotypes: Japan/Thailand and Korea (Anderson and Trueman, 2000)

General

It is well documented that there are two phases in the life cycle of *V. destructor* females: a phoretic phase (on adult bees) and a reproductive phase (within the sealed brood cells) (Rosenkranz et al., 2010).*Varroa* females are transported on the adult bees and they can be spread by foraging and swarming bees, as well through moving around frames of sealed brood.

The mites suck hemolymph from both the adult bees and the larvae/pupae within the sealed cells.For reproduction, the female mites enter the cells of drone or worker larvae 2 days or 1 day before capping respectively (Boot et al., 1992).The first, is an unfertilized egg and it will develop into an haploid male, while all other eggs are fertilized and are developed into females (Ifantidis, 1983; Martin 1994, 1995a).The amount of brood throughout the season, the percentage of drone brood, the swarming tendency, and the broodless periods during winter or during dry seasons have a great impact on the reproduction of the *Varroa* mite.

Varroa reproduction phase

The invasion rates depend on the number of the initial phoretic mites and the number of brood cells suitable for invasion (i.e. 1 or 2 days before capping) (Boot et al., 1995; Martin 1998, 2001; Calis et al., 1999a,b). Shortly after leaving the brood cells, the mites preferentially infest nurse bees (Kraus, 1993; Kuenen and Calderone, 1997). The mites invade the drone cells with 8–10 times more compared to worker brood (Fuchs, 1990; Boot et al., 1995; Calderone and Kuenen, 2001).

Brood cells of European honeybees are in general more highly infested than cells of Africanized bees (maybe because they are slightly smaller) (Message and Goncalves,1995; Piccirillo and De Jong, 2003).Martin (1994, 1995b) calculated that the reproduction rate is 1.3–1.45 in single infested worker brood and, due to the longer capping period, 2.2–2.6 in drone brood. The *Varroa* female can have between 2 and 3 reproductive cycles (Fries and Rosenkranz, 1996; Martin and Kemp, 1997).

In European honeybee subspecies, about 5–20% of the mites remain infertile (Rosenkranz and Engels, 1994; Martin, 1994, 1995a; Martin et al., 1997; Rosenkranz, 1999; Garrido et al., 2003; Al Aattal et al., 2006). For example, Donze et al. (1996) and Martin et al. (1997) it is shown that in 11–21% of the brood cells there is no male varroa. According also to Martin (1998) the adjusted mean percentages of mites producing viable female offspring are 69.8% in worker and 74.7% in drone brood. In multiple invaded drone and worker brood cells, the reproductive rate per female mite is significantly reduced as there is a density factor of 0, 1, 0.91, 0.86 and 0.60 for 0, 1, 2, 3 and 4 mites, respectively (Fuchs and Langenbach, 1989; Martin, 1995b; Martin and Medina, 2004; Mondragón et al., 2006).

Significant correlations have been shown between the amount of brood and/or the fertility of the mites and the population growth of the mites (Arechavaleta- Velasco and Guzman-Novoa, 2001; Lodesani et al., 2002; Harris et al., 2003). However, although the age of a queen is negatively correlated to the amount of brood and population in a colony, the infestation rate on workers and drones has been shown at least in one case to be positively correlated (Akyol et al., 2007).

Phoretic phase

The mites are released when the adult bee is emerging or if a dead pupa is removed and the mite becomes phoretic (Oldroyd, 1999). The phoretic mites prefer young worker bees (Kuenen and Calderone, 1997). However, on the day of emergence, mites have a high risk of falling from the comb and dying (Martin and Kemp, 1997).

Varroa Mortality

The natural daily mortality of phoretic mites depends on the season (broodright or broodless periods). When brood is present, the mortality risk for a mite is about to 0.006 (according to Martin 1998 and Fries et al., 1994).When no brood is present, then the mortality risk for a mite is about 0.002, 0.004 or 0.0012 according to Moosbeckhofer (1991), Fries et al. (1994) and Martin (1998), respectively. The latter originates from a larger set of data.

However, another type of mite mortality is the one caused by *Varroa* treatment and depends on the type and duration of the treatment as well as the mite resistance to the agent (see the review of Rosenkranz et al., 2010). However, there is no *Varroa* treatment available which fulfills both the criteria 'safe and effective 100%'. It has also been suggested that under temperate conditions untreated colonies may collapse in 3–4 years after the initial infestation (Korpela et al., 1992; Büchler, 1994).

Varroa destructor has already become resistant to tau-fluvalinate (Milani, 1994; Sammataro et al., 2005) as well as to other pyrethroids like acrinathrin and flumethrin, and organophosphates like coumaphos and the formamidine amitraz (Lodesani et al., 1995; Trouiller, 1998; Elzen et al., 1999a,b; reviewed in Milani, 1999).

Parameters influencing *Varroa* reproduction and development

It also has been shown that honeybees exhibit two special behavioral skills, called 'grooming behavior' and 'removal of parasitized brood cells' (hygienic behavior) which might impair the survival of *V. destructor* (reviewed by Boecking and Spivak, 1999; Evans and Spivak, 2010). Moreover, *A. cerana* exhibits another unique behavior (of entombing the parasitized drone brood) which prevents the hatching of these cells and kills the invaded mites (reviewed by Rath, 1999).

Reproductive rates are reduced at temperatures above 36.0°C (Le Conte et al., 1990; Kraus et al., 1998) and they are higher at 70 relative humidity (RH) % than at 40 RH % (Le Conte et al., 1990). *Varroa* mites stop reproducing when relative humidity in the broodnest exceeds 80 RH % (Kraus and Velthuis, 1997) and they begin to die when temperatures exceed 38.0°C (Le Conte et al., 1990).

Swarming can also reduce the mites in the colony as a big proportion of them depart with the swarm. Martin 1998 showed that 65% of the *Varroa* mites are still in the brood of the mother colonies.

Damages caused at individual level

A hatching bee has a reduced body weight due to the loss of hemolymph during the sealed brood stage. De Jong et al. (1982) showed that even a single infestation results in a 7% average loss of body weight. The reduction in adult weight can be 30% if the immature bee is infested by more than 3 mites; however the effect is less pronounced in drone than in worker bees (De Jong et al., 1982).

Especially drones, lose 11–19% of their body weight depending of infestation rate (Duay et al., 2003), which led to decreased flight performance (Duay et al., 2002).

Other effects: when worker bees are parasitized during their development, they start foraging earlier and have a reduced life span (De Jong et al., 1982; Schneider and Drescher, 1987; Amdam et al., 2004). *Varroa* parasitized foragers also have a reduced ability to navigate (Ruano et al., 1991) and eventually a lower rate of return to the colony (Kralj and Fuchs, 2006; Kralj et al., 2007). .

Varroa transmit viruses, which also cause problems to bees, especially the deformed wing virus (DWV) and the acute bee paralysis virus (ABPV). DWV is strongly associated with winter colony mortality (Highfield et al., 2009; Genersch et al., 2010). The DWV infection is causing the typical symptoms of crippled wings and shortened abdomen when the colonies are heavily infested (Boecking and Genersch, 2008; de Miranda and Genersch, 2010). The morphological deformities (small body size, shortened abdomen, deformed wings), also reduce vigour and longevity, flight duration and the homing ability of foragers (Schneider and Drescher, 1987; Koch and Ritter, 1991; Romero-Vera and Otero-Colina, 2002; Garedew et al., 2004; Kralj and Fuchs, 2006).

Additionally, it is believed that *Varroa* mites can induce immunosuppression in parasitized pupae and activate covert virus infections (Yang and Cox-Foster, 2007). DWV may also affect aggression (Fujiyuki et al., 2004) and learning behaviors of adult bees (Iqbal and Muller, 2007).

For ABPV, one billion viral particles of ABPV are needed to cause death *via* ingestion, but only 100 virus particles will cause death (Genersch and Aubert, 2010) when vectored by *Varroa* and injected into the developing bee's hemolymph. According to Bailey (1965) bees infected with ABPV virus die within 8 days.

More than 75% of the egg samples have been found infected with at least one virus (Ravoet et al., 2015). The most abundant viruses were DWV and the Sacbrood Virus, although the Lake Sinai Virus and ABPV were also occasionally detected (Ravoet et al., 2015). In the same study, it was shown that carnica bees were less infected than Buckfast bees; they had a lower average total number of viruses.

Viruses can spread from an infected mite to a bee pupa or adult bee and then to uninfected mites (Bowen-Walker et al., 1999, Martin 2001, Martin et al., 2010). It is possible that in heavily *Varroa* infested colonies, almost 100% of adult workers are infected by DWV, even without showing symptoms (de Miranda et al., 2012). After treatment a gradual decrease in virus titers occurs as infected bees are replaced by healthy ones (Martin et al., 2010).

The probability that an infected mite will infect a healthy pupa is 0.89 for DWV and 1 for ABPV. Then the probability that an infected pupa will infect a new healthy mite is 1 and 0, respectively, as pupae infected with ABPV always die. The probability that an infected pupa will die of the DWV virus is 0.2 and daily mortality rate of in-hive bees and foragers infected as adults is 0.2 (Martin, 2001).

Damages at the colony level

At the colony level the damage caused is probably due to a significantly lower chance of infected drones to mate (Duay et al., 2002) and/or because the parasitized colonies produce less swarms (Fries et al., 2003; Villa et al., 2008).The decline in emerging bees due to ABPV larvae death, causes a colony to collapse. A colony infected with an ABPV epidemic will die within one season (Sumpter and Martin, 2004).

Low infestations remain undetected. Moderate infestations may reduce the growth of the honeybee population and, therefore, the honey yield, but the clinical symptoms (viruses' damage) may still not be evident. However, when the host population is decreasing while the mite population still increases during fall, the damaging evidence at the colony level is particularly apparent (Fries et al., 2003).

The damage threshold depends on the bee and brood population, the season and the presence of bee viruses. According to and Liebig (2001) and Rosenkranz et al. (2010), under the environmental conditions found in Germany, an infestation rate of the winter bees of more than 7% may lead to colony collapse. Similarly, Delaplane and Hood (1999) suggested an economic threshold of 3000–4000 mites per colony for the Southern United States of America (USA). However, according to Fries et al. (2003) and Rosenkranz et al. (2006), if the infestation rate in the adult bees during summer exceeds 30%, the colony does not have a chance to survive the following winter.

Colony mortality was strongly and positively related with the presence of CBPV, DWV, ABPV, SBV and BQCV viruses per apiary. Colony mortality can be almost 30% higher (Nguyen et al., 2011). ABPV virus facilitates a co-infection with other viruses and can cause a reduction in the bee lifespan (Kovac and Crailsheim, 1988; Amdam et al., 2004).

Amdam et al. (2004) suggested that workers infested by *Varroa* during pupa stage fail to develop key physiological characteristics of long-lived winter bees, and this may not allow them to survive until spring.

Interactions-Synergistic effects

There are only a few reports which associate *Varroa* with other stressors, including *Paenibacillus larvae,* the causative agent of American foulbrood (Brødsgaard et al., 2000), tracheal mite (Downey and Winston, 2001). Similarly, Bernal et al. (2011) suggested that the combination of *V.destructor* and *N. ceranae* could augment the risk of colony death and Hedtke et al. (2011) showed that infestation of honeybees by *Varroa* may induce chalkbrood outbreaks in summer.

In addition, few evidences show a link between *Varroa* and pesticides. Infestations of *Varroa* mites were significantly higher in exposed colonies to imidacloprid (Dively et al., 2015).

Nosema spp.

General

Nosema spp are Microsporidia, some fungi-related obligate intracellular parasites. The western honeybee (*Apis mellifera*) can be parasitized by two *Nosema* species, *N. apis* and the emerging *N. ceranae*. Both microsporidian species are the etiologic agents of nosemosis, one of the most widespread diseases of the adult honeybee (Fries, 2010; Higes et al., 2010). *N. ceranae* has become the predominant microsporidian species infecting *Apis mellifera* and is now considered as a major threat to the western honeybee at both the individual and colony levels (Fries, 2010; Vanengelsdorp and Meixner; 2010; Higes et al., 2013).

Faecal-oral and oral-oral (by trophallaxis) are the main routes of transmission between bees (Smith, 2012). Then the parasites invade and develop within the cytoplasm of the epithelial cells of the adult honeybee midgut (Fries, 2010).

Although *N. apis* infections are often associated with dysentery, such symptom are not observed for *N. ceranae*-infected colonies (Higes et al., 2008a).

The large survey conducted in North-Eastern Germany in 22 apiaries (220 colonies) during 6 years (Hedtke et al., 2011) showed that the prevalence of *Nosema* was higher in spring (18.9% *N. apis* and 14.46% *N. ceranae*) than in autumn (5.84% *N. apis* and 3.02% *N. ceranae*). Low levels of mixed *N. apis* - *N. ceranae* infections were found (0.38% in autumn; 5.26% in spring).

Targets

Nosema apis and *N. ceranae* can infect all adult bees: workers, drones (Traver and Fell, 2011) and queens (Higes et al., 2009; Alaux et al., 2011; Roberts et al., 2015).

Effects of *N. ceranae* on honeybees (behavior and physiological traits)

- **Effects on mortality**

Nosema shortens bee lifespan (Higes et al., 2006; Alaux et al., 2010; Vidau et al., 2011; Goblirsch et al., 2013). High mortality rates that can reach 93% at 7 days post-infection have been described after inoculation of bees with 100,000 to 125,000 spores of *N. ceranae* (Higes et al., 2007; Martín-Hernández et al., 2011). In some cases, all infected bees died between two and three weeks after the infection (Higes et al., 2008a; Dussaubat et al., 2012, 2013a). In contrast, other studies using the same infectious doses (100 000 spores/bee) revealed much lower mortality rates, ranging from only 30% (Alaux et al., 2010) to 44% (Paxton et al., 2007). Honeybee survival may be dependent on the infectious dose, i.e. survival decreases when the infectious dose increases (Martín-Hernández et al., 2011). In contrast, Porrini et al. (2011) did not observe any dose-dependent effect.

- **Effects on nutrition (sugar consumption)**

The honeybee midgut is involved in immunity, digestion and detoxification, it is also the primary site of *N. ceranae* proliferation and can, thus, be impaired by this parasite leading to disorders of the digestive function (Higes et al., 2007; García-Palencia et al., 2010; Dussaubat et al., 2012). The pathology induced by *N. ceranae* development was also shown to be characterized by an impairment of the enteric nervous system of the gut that contributes to the regulation of feeding and swallowing and gut peristalsis and metabolism.

Analysis of the metabolite profiling of hemolymph from *N. ceranae*-infected honeybees revealed that the parasite can cause a severe nutritional and energetic stress. The majority of free amino acids are present at lower concentrations in the hemolymph of infected honeybees (Aliferis et al., 2012). The infection also results in a significant decrease in the levels of many carbohydrates, which are the main energy source in the honeybee (Aliferis et al., 2012). For example, the foragers infected with *N. ceranae* have diminished hemolymph trehalose concentrations; at least two times lower than in uninfected individuals (Mayack and Naug, 2010).

The energetic stress induced by *N. ceranae* seems to result in an elevated hunger, bees consume more sucrose when infected (Mayack and Naug, 2009; Alaux et al., 2010; Martín-Hernández et al., 2011). Parasitized bees are also less willing to share food with their nest mates (Naug and Gibbs, 2009). The lifespan of infected bees is significantly reduced when they are fed with limited amounts

of sucrose but is almost to the same extent as uninfected ones when they were fed with ad libitum sucrose (Mayack and Naug, 2009). This suggests that energetic stress is the probable cause of the shortened life span observed in infected bees.

- **Effects on foraging activity**

The energetic stress induced by *N. ceranae* may influence foraging behavior (flying ability) of infected bees (Mayack and Naug, 2010). Homing success was significantly reduced in *Nosema*-infected (65.8%) versus healthy foragers (92.5%) (Wolf et al., 2014).

Infection by *N. ceranae* may reduce homing and orientation abilities in the honeybee (Kralj and Fuchs, 2010) but also lead to compensation effects such as increased flight activity (higher number of exits/bee/day) than uninfected bees (Dussaubat et al., 2013b).

In addition, and linked to the hormonal titers involved in temporal polyethism, *N. ceranae* may modify and advance behavioral maturation in workers. Infected bees were nearly twice as likely to engage in precocious foraging and lived 9 days less, on average, compared to controls (Goblirsch et al., 2013; Lecocq et al., 2016). Bees co-infected by both *N. apis* and *N. ceranae* are characterized by alteration of hormonal pathways linked to behavioral maturation in workers. Further description of effects of *N. ceranae* on pheromones production and behavioral maturation is provided by Dussaubat et al. (2010, 2013b), Alaux et al. (2011), Holt et al. (2013), Mayack et al. (2015).

- **Modulation of virulence**

Both quality and diversity of pollen diets would be important factors for bee survival when infected by *N. ceranae* with increase survival in bees infected with *N. ceranae* fed with pollen-enriched diet (Porrini et al., 2011; Di Pasquale et al., 2013; Jack et al., 2016) and nutritional supplements (Fleming et al., 2015). Bees fed with polyfloral pollens lived longer than bees fed with monofloral pollens (Di Pasquale et al., 2013).

Virulence could also be attributed to parasite variants according to geographic origin (Fries, 2010; Chaimanee et al., 2013; Higes et al., 2013), but it needs confirmation (Dussaubat et al., 2013a). The virulence of *N. ceranae* should be related to an alteration of the host-parasite interactions in response to environmental cues, including environmental stressors (Bromenshenk et al., 2010; Gisder et al., 2010; Chen et al., 2012), the honeybee genetic background (Fontbonne et al., 2013), rather than to its polymorphism.

- **Links between *Nosema* and viruses**

Some studies revealed associations between viruses and *Nosema* spp (Bailey et al., 1983; Bromenshenk et al., 2010; Toplak et al., 2013; Doublet et al., 2015a).

Synergistic effects were reported:

  - Between *N. apis* and filamentous virus (FV), beevirus Y (BVY) and black queen cell virus (BQCV) (Bailey et al., 1983).
  - Between *N. ceranae* and BQCV: at 11 days post-infection, 50% of the co-infected workers honeybees were dead, while only 20% of N. *ceranae*-infected bees and less 5% of BQCV infected and control honey bees were dead (Doublet et al., 2015a).
  - Between *N. ceranae* and CBPV: co-infection of workers with CBPV and *N. ceranae* increased replication ability for CBPV and induced high mortality rates (Toplak et al., 2013).

No interactions were reported between:

  - *Nosema ceranae* and IAPV (Cox-Foster et al., 2007);
  - *N. ceranae* and DWV (Martin et al., 2013). However, recently, Zheng et al. (2015) show synergistic interactions.

Antagonist interactions were observed between *N. ceranae* and DWV (Costa et al., 2011; Doublet et al., 2015b).

- Finally, some studies reported lower survival in bees and higher risks of colony loss when bees were exposed to:Mix *Nosema* spp. (*N. apis* and *N. ceranae*) infection (Milbrath et al., 2015) which resulted in higher number of spores on bees than in single infections;
- *N. ceranae* with *Varroa destructor* (Bernal et al., 2011);
- *N. ceranae* with the trypanosome *Crithidia mellificae* (Ravoet et al., 2013; Schwarz and Evans, 2013).

- **Links between *Nosema* and pesticides**

N. ceranae can sensitize the honeybees to chemical stressors (Alaux et al., 2010; Vidau et al., 2011; Aufauvre et al., 2012, 2014; Pettis et al., 2012, 2013; Wu et al., 2012; Retschnig et al., 2014). Here below some examples of laboratory and field studies showing such effects:

- Increased susceptibility to organochlorine or organophosphate pesticides Dichlorodiphenyltrichloroethane (DDT) in bees infected by *N. apis* (Ladas, 1972).
- Increased mortality rate in bees with synergistic interactions between *Nosema* spp. and imidacloprid (Alaux et al., 2010).
- Increased mortality rate in bees with synergistic interactions between *N. ceranae* and sublethal exposures to thiacloprid or fipronil (increase of spore number with thiacloprid, decrease with fipronil) (Vidau et al., 2011).
- Synergistic interactions on bee mortality between sublethal doses of thiacloprid and *N. ceranae* (Retschnig et al., 2014).
- Increased mortality rate in bees infected with *N. ceranae* and exposed to fipronil or imidacloprid (Aufauvre et al., 2014).
- Increased mortality rate in adult bees with synergistic interaction between *N. ceranae* and sublethal exposures to thiacloprid (Doublet et al., 2015a).
- Increased susceptibility to *Nosema* infection when bees are pre-exposed to imidacloprid (Pettis et al., 2012; Wu et al., 2012).

- **Other links**

Effects are also observed on pheromone production in worker and queen honeybees (Dussaubat et al., 2010; Alaux et al., 2011) and on bee immune response (Antúnez et al., 2009; Alaux et al., 2010; Chaimanee et al., 2012; Dussaubat et al., 2012; Aufauvre et al., 2014).

Appendix C - Information on Beekeeping Management Practices (BMP)

BMP: 'Change in the number of workers'		
Model component		
C-HP modules	**Description of effects**	**Additional information (references)**
Demographic traits	Alterations of the demographic traits are quantified by the number of adult bees added or removed. Fecundity is not altered. In the donor colony, development of brood may be impaired if the ratio bees to brood is too low (< 0.5). Also life-span of the adult bees may decrease when the bees feed more Larvae (Woyke, 1984).	
Scenario component		
Biological Agents module	**Description of effects**	**Additional information (references)**
Varroa destructor DWV ABPV *Nosema* spp.	The increased number of immature/adult bees in the receiver colony has beneficial effects on the control of biological agents. However, the introduced bees may carry biological agents that could spread and develop within the receiver colony, increasing their prevalence/infection rate. The increased infection rate/prevalence in the receiver colony depends on the infection rate/prevalence (quantitative information would be informative) of the biological agents within the added bees.	Bailey L and Ball BV, 1991 Fries, 1993
BMP: 'Chemical control'		
Model component		

C-HP modules	Description of effects	Additional information (references)
Demographic traits	Chemical agents used in apiculture could elicit adverse side-effects on honeybees. Coumaphos and tau-fluvalinate show chronic oral toxicity to honeybee larvae, alone and in combination. The larval mortality is over than two-fold compared to non-exposed larvae (Zhu et al., 2014) Queens reared in miticide-laden beeswax (coumaphos and fluvalinate) showed significantly lower sperm counts and viability, and higher mating frequency, compared to queens reared in miticide-free beeswax (Rangel and Tarpy, 2015) **Fluvalinate** Reports of LD_{50} of fluvalinate have been increasing since its first use in the 80s: from 65.85 µg/bee for honeybees (Atkins et al., 1981) to 0.23 µg/bee reported by the US-EPA Office of Pesticide Programs (US-EPA-OPP) (US-EPA-OPP, 2005). Toxicity of tau-fluvalinate in combination with other acaricides increases. Interactions between tau-fluvalinate and coumaphos may occur because these acaricides interact with detoxicative enzymes (Johnson et al., 2013). **Amitraz** Amitraz has shown acute toxicity in larvae showing increased apoptotic cell death in the midgut (Gregorc and Bowen, 2000). Sublethal amitraz pre-treatment increases the toxicity of the three P450-detoxified acaricides, but amitraz toxicity does not change by sublethal treatment with the same three acaricides. In combination with other acaricides, amitraz toxicity is mostly unchanged (Johnson et al., 2013). **Coumaphos** Effects on queens: Mortality of developing queens. Sublethal effects such as lower weight, lower ovary weight, physical abnormalities and atypical behavior (Haarmann et al., 2002). Mortality of 100% developing queen larvae at 1000 mg/kg, and more than 50% at the 100 mg/kg concentration. Additionally, queens that survived exposure to 100 mg/kg coumaphos weighed significantly less than control queens (Pettis et al., 2004) Effects on workers: Delayed development was observed in bees reared in treatment combs containing high levels of pesticides (mostly coumaphos and fluvalinate) particularly in the early stages (day 4 and 8) of worker bee development. Adult longevity was reduced by 4 days in bees exposed to pesticide residues in contaminated brood comb during development (Wu et al., 2011)	Dose–response curves and LD_{50} for workers and queens to several acaricides are given in Dahlgren et al., 2012 LC_{50} in six-day-old bees were determined for the following acaricides: tau-fluvalinate, flumethrin, amitraz and coumaphos (Garrido et al., 2013) LC_{50} were: 0.1 µg/dish for amitraz; 0.29 µg/dish for fluvalinate; 0.34 µg/dish for flumethrin; and 0.57 µg/dish for coumaphos, respectively (Maggi et al., 2008) LC_{50} baseline levels of amitraz, coumaphos, fluvalinate and flumethrin in populations of *Varroa destructor* from Buenos Aires Province, Argentina Aliano et al., 2006 Ebert et al., 2007

	Thymol Alteration of metabolic responses (detoxification gene expression pathways, components of the immune system responsible for cellular response and developmental genes), which could potentially interfere with the health of individual honeybees and entire colonies. Thymol treatment can induce brood removal and may result in increased queen mortality (Whittington et al., 2000; Floris et al., 2004). **Formic acid, Oxalic acid** If applied according to GBP, no toxic effects.	
Behavioural traits	Increased ventilation when essential oils (thymol) are applied.	
Physiological traits	Treatments with coumaphos and fluvalinate cause significant changes in genes involved in detoxification, behavioral maturation, immunity and nutrition (study on genome-wide gene expression patterns of honeybee workers) (Schmehl et al., 2014) Following treatments with coumaphos, alteration of metabolic responses (detoxification gene expression pathways, components of the immune system responsible for cellular response and developmental genes) were observed, which could potentially interfere with the health of individual honeybees and entire colonies (Boncristiani et al, 2012)	
In-hive products	Chemical treatments are typically applied inside the hive and those molecules are therefore often found as residues in various bee matrices such as wax, beebread and honey. Most acaricides are lypophilic, thus the matrix with highest risk of residues is beeswax. If residues in beeswax are high the molecules may then pass into the stored honey. Contamination may also be indirect, i.e. from old wax to new wax. Treatment with amitraz can lead to residues in honey. Oxalic acid and thymol may lead to residues in honey if not applied according to GBP.	Floris et al., 2001 Bogdanov, 2006 Martel et al., 2007 Lodesani et al., 2008 Mullin et al., 2010
Scenario component		
Biological Agents module	**Description of effects**	**Additional information (references)**
Varroa destructor DWV ABPV *Nosema* spp.	Chemical agents used in apiculture act on biological agents leading consequently to beneficial effects on honeybee health by reduction of impact from diseases. Chemical agents are used by many beekeepers throughout Europe (e.g. acaricides for *Varroa* control). Application of chemical treatments ensures control of the *Varroa* mite population. If applied correctly, the efficacy of the authorised products reaches 99% (Oxalic acid: Rademacher and Harz, 2006). Successful control of *Varroa* limits replication and transmission of DWV and ABPV (see Le	

	Conte et al., 2010; Nazzi et al., 2012; Emsen et al., 2015)	
Pesticides module	**Description of effects**	**Additional information (references)**
Contact and oral exposure	Treatment implies exposure of the bees to a pesticide inside the colony, usually by direct contact. Acaricides are often applied as impregnated strips which are left in the colony for some time (e.g. commercial formulations of tau-fluvalinate and amitraz). Chemical treatment residues can end up in food stores, thus bees can also be exposed to the active ingredients orally.	
BMP: 'Replacement of combs with brood'		
Model component		
C-HP modules	**Description of effects**	**Additional information (references)**
Demographic traits	Combs with brood derived from one colony and inserted into another colony will result in (i) an increase the colony strength in the receiver colony once the brood emerges and (ii) a reduction in colony strength in the donor colony. **Receiver colony:** Increased demography: increase of brood (and adult bee population, if present) proportional to the quantity present in the added comb. **Donor colony:** Alteration of demography: decrease of brood (and adult bee population, if present) proportional to the quantity present in the added comb.	
Behavioural traits	Unbalanced numbers of immatures/adult bees may lead to negative consequences to the receiver colony. Brood rearing requires time and energy from nurse bees (i.e. royal and worker jelly production, feeding and thermoregulation effort). Thus, it is important to know the amount of unsealed (higher energy requiring) and sealed (lower energy requiring) brood being transferred to the receiver colony. **Receiver colony:** Effects in the short-term: Possible task modification of bees which become nurses and need to take care of the brood (i.e. increased thermoregulation, brood care and nursing); indirect effects on energy requirements from nurses (in terms of time, food due to increased number of bees nursing) Direct effect on foraging activity that is increased because of the need of more food (for the	Filmer, 1932 Allen and Jeffree, 1956 Al-Tikritya et al., 1972 Eischen et al., 1982, 1983,1984 Winston and Punnett, 1982 Winston and Fergusson 1986 Pankiw et al., 1998 Le Conte et al., 2001 Pankiw, 2004 Pankiw et al., 2004

	brood) Magnitude of the effects is related to the quantity of brood (added and present in the colony), number of total adult bees, number of adult nurses, quantity of food available (pollen and nectar), climate and weather. Increase of brood pheromone production (consequence on foraging, especially pollen foragers). <u>Effects in the long-term</u> (from the moment the brood emerges; we assume that the receiver colony manages to rear the brood appropriately): Alteration of demography: increase of adult bees, possible task modification of bees that used to be nurses and now can do other tasks. **Donor colony:** Consequence of reduced quantity of brood: Possible task modification of bees that used to be nurses and now can do other tasks: e.g. increase of foraging, comb building decrease of brood pheromone production	
In-hive products	Food could be stored in the moved comb. The quantity and quality of food present in the comb should be recorded, since it influences the nutritional status of the colony: **Receiver colony:** • increase of food stored **Donor colony:** • reduction of food stored	
Scenario component		
Biological Agents module	**Description of effects**	**Additional information (references)**
Varroa destructor DWV ABPV *Nosema* spp.	Brood may contain infectious agents and pests (e.g. Varroa mites, *Nosema* spp., etc) that may spread within the new colony. Bacterial diseases infect immature stages and thus may be present in the brood. The Varroa mite reproduces in brood and thus may also be present, together with its associated viruses. Other viruses may be present (SBV, CBPV). If the donor colony was infected by *Nosema* spp., faeces containing infectious spores may be present on the combs.	Bailey and Ball BV, 1991 Fries, 1993 Lindström et al., 2008. ApeNet, BeeNet, Epilobee monitoring projects
Pesticides module	**Description of effects**	**Additional information (references)**
Contact and oral exposure	Possible exposure if the comb contained contaminated wax or food, either in the brood cells or stores	

BMP: 'Replacement of combs with feed sources'		
Model component		
C-HP modules	**Description of effects**	**Additional information (references)**
Demographic traits	Removal or introduction of combs with feed, if covered by adult bees, will alter colony demography in relation to the number of adult bees present on the comb: **Receiver colony:** Increased demography: increased number of adult bees (if present) proportionally to the number of adult bees present on the added comb. **Donor colony:** Reduced demography: decreased number of adult bees (if present) proportionally to the number of adult bees present on the added comb.	
Behavioural traits	Comb building: the timing of comb building is influenced by two variables that need to occur simultaneously: nectar availability/influx and stored food quantity/comb fullness (> 80%). The conditions required to initiate comb building are different from those required to maintain the building. To continue comb building, the honeybee needs to continue experiencing a high nectar influx (Seeley, 1995).	Kelley, 1991
In-hive products	The introduction of combs with feed sources is essential when food resources (i.e. nectar availability) and/or food stores are low. Quality and quantity of the food introduced alters the nutritional status of the colony (i.e. in-hive products), increasing (receiver colony) and decreasing (donor colony) the quantity of in-hive products. The specific quantity and quality of food present in the comb should be recorded. The quantity of in-hive products (e.g. wax) contained in a comb, on average, depends on the size of the frame (i.e. type of hive used).	Dadant, 1975 Seeley and Morse, 1976
Scenario component		
Biological Agents module	**Description of effects**	**Additional information (references)**
Varroa destructor	The introduction of food combs carries risks related to the possible contamination by	Fries, 1993

DWV ABPV *Nosema* spp.	infectious agents (e.g. *Paenibacillus larvae*, *Nosema* spp.) that are present in the food stores.	Lindström et al., 2008 ApeNet, BeeNet, Epilobee monitoring projects
Pesticides module	**Description of effects**	**Additional information (references)**
Contact and oral exposure	The introduction of food combs carries risks related to the possible contamination by pesticides (i.e. both miticides and agrochemicals). To define them, quantity of contaminated food and concentration should be known. Chemical treatment residues can end up in food stores, thus bees can also be exposed to the active ingredients orally.	Highest contamination levels could be assessed through a thorough analysis of the published data, for each active ingredient. Monitoring projects such as ApeNet, BeeNet, Epilobee, could provide relevant information. Johnson et al., 2010 Mullin et al., 2010 Codling et al., 2016
BMP: 'Beekeeper category and experience'		
Scenario component		
C-HP and Biological Agents modules	**Description of effects**	**Additional information (references)**
	The beekeeper (BK) experience/category is linked to personal beekeeping skills gained through practice and training. The BK experience/category influences the ability to understand and cope with the health status of the colonies. It also influences the BMP used in specific scenarios. For example, this factor is related to the probability of recognising symptoms of disease and of taking appropriate measures. Therefore, assuming that BK follow the GBP does not guarantee that equal consequences will be obtained at colony level. Thus, BK experience/category would be relevant to reduce the variability caused by the quality of the practice. Epilobee 2012-2014: beekeepers were characterised based on the number of colonies and apiaries being managed, the income generated by the activity of the BK, the BK experience (i.e. for how long he/she has been a beekeeper, if he/she is a member of a BK association, if he/she has a qualification in bee husbandry, if he/she was trained), and whether the BK had	EC, 2004 EC, 2014

	an apiarist/medicine book. The analysis of these variables is presented in Jacques et al. (2016). COLOSS assessed the number of managed apiaries by BKs. EC (2013): focus on years of experience of the beekeepers. According to the Reg. (EU) N. 917/2004, two types of BK are distinguished, professional beekeepers with more than 150 hives, and non-professional beekeepers with less than 150 hives. However in Europe, there was a common consensus amongst countries on three beekeeper categories: hobby, part-time and professional BK (Chauzat et al., 2013). According to the Reg. (EC) No 834/2007 and Reg. (EC) No 836/2014, the BK production system is either 'Conventional' or 'Organic'.	
BMP: 'Supplementary feeding'		
Model component		
C-HP modules	**Description of effects**	**Additional information (references)**
Demographic traits	In colonies provided with supplemental feeding, brood production and honey yield are increased, most likely due to increased egg laying and increased worker bee longevity. Longevity tests performed with bees held in hoarding cages show that bees collected from colonies which were provided with extra pollen have a significantly higher survivorship. If the supplementary feeding is provided in period of dearth, it can increase the longevity of adult bees and reduce the mortality of immatures, which may otherwise be cannibalised.	Schmidt et al., 1995 Dodologlu et al., 2004 Mattila and Otis, 2006 Somerville and Nicol, 2006 Dodologlu and Emsen, 2007 van der Steen, 2007 DeGrandi-Hoffman et al., 2008
Physiological traits	Honeybees fed with sugar syrup alone have lower protein concentrations and smaller hypopharyngeal glands compared to bees fed diets containing proteins, with the other feeding treatments especially as the bees aged (DeGrandi-Hoffman et al., 2010). The virus concentrations increase as honeybees age and are highest when bees are fed with sugar syrup alone and lowest in bees fed a diet containing pollen (DeGrandi-Hoffman et al., 2010). Overall results from DeGrandi-Hoffman et al. (2010) show that there could be a connection between diet, protein levels and immune response and indicate that colony losses might be reduced by alleviating protein stress through supplemental feeding.	
Scenario component		

Biological Agents module	Description of effects	Additional information (references)
Varroa destructor DWV ABPV *Nosema* spp.	Food nutritional quality (especially related to pollen quality) and pollen quantity could influence bee susceptibility to stressors (Di Pasquale et al., 2013), such as *N. ceranae*. Colonies provided with supplementary pollen during summer have lower levels of *Nosema* infection in the following spring (Lodesani et al., 2012)	
Pesticides module	**Description of effects**	**Additional information (references)**
Oral exposure	The food/ingredients used in the supplementary feeding could be contaminated by toxic nutrients and pesticides, and could therefore impair bee health.	Barker, 1977