1.4 Potential routes of exposure as a foundation for a risk assessment scheme: a Conceptual Model

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Abstract

Background: The global interest in improving the regulatory risk assessment of pesticides in honeybees and other pollinator insects has led to new test requirements and a conceptual model has been published in the US. It is of interest for modellers and risk assessors to have a more detailed conceptual model that describes the movement of deleterious substances from the point of initial exposure to the point of impact on the protection goals, such as colony health, or honey production.

Results: The flow of pesticide residues from application to distribution in the hive is described in an integrated conceptual model. The significance of this model for assessing the relative contribution of various potential routes of exposure, guiding test requirements and describing the quantitative distribution of residues among the castes and task groups of honeybees in the colony was described using data from studies with chlorpyrifos and several neonicotinoids.

Conclusion: The quantitative pollinator conceptual model (QPCM) describes the flow pathways and potential exposure routes for honeybees and other bee pollinators in sufficient detail to support quantitative exposure modelling and risk assessment and shows the importance of measuring the distribution of pesticide residues in the areas that lead to exposure and in the hive.

Key words: Honey bee, pollinator, risk assessment, conceptual model

1. Introduction

In the past, the risk assessment for pollinators has been based mainly on evaluation of toxicity to individual insects, usually represented by honeybees (*Apis mellifera*). There is widespread interest in improving the risk assessment methodology for pollinators and particularly for honeybees by taking into consideration more details of species specific behavior and biology. (1) The computational modelling of honeybee social behavior is also advancing with the development of the BEEHAVE model. (2) Meanwhile conceptual models which describe the network of potential routes of exposure of bees and other insect pollinators to pesticides have also recently published. (3),(4) _ENREF_2 This work presents a more advanced version of the conceptual model for pollinator risk assessment, which includes both exposure inputs, and depurition over time.

2. Results

A refined version of the Quantitative Pollinator Conceptual Model (QPCM) was developed based on field studies with chlorpyrifos and neonicotinoids which are described elsewhere, (5) (Purdy 2014, ACS Poster San Francisco). The development of this model is described below in terms of problem formulation, scope, pollinator biology, routes of exposure and depurition, cofactors, and quantitive risk assessment.

2.1 Scope and Problem Formulation

The use of a conceptual model in risk assessment begins with a problem formulation statement. For the present work it was: “Is there sufficient exposure of pollinators to pesticides and/or their degradates, to present a risk of widespread and repeated mortality or biological impairment to individuals or populations of pollinators?” It is also essential to consider which

cofactors must be considered. Examples include: nutrition, pest/disease, beecare, agronomy, climate, genetics.

There are thousands of kinds of bee pollinators. Not all are present in an agro-ecosystem.

For example many *Andrena* and *Halictid* species focus on non-crop floral species with a short growth season. Of those present, many are less exposed to pesticides with no indication of greater sensitivity. The scope of the conceptual model for evaluating potential effects of agro-chemicals on pollinators was defined as Agro-ecosystems in which pesticides are used and potentially impacted areas connected to them, which contain managed and wild pollinators. European and US regulations have defined three major groups of pollinators to be assessed. Honeybees, bumblebees and solitary bees.(6) Honeybees have long been used as surrogates for other pollinators in such tests as acute oral and contact toxicity. In the following discussion of the risk assessment model, the focus is on honeybees, with comments on the applicability to other species. While honeybees are the most studied bee species, they are also the most complex, and essential aspects of honeybee biology tend to be overlooked, particularly when extrapolations are made from laboratory tests on individual bees.

2.2 Pollinator Biology in Risk Assessment

The critical aspects of honeybee biology include the eusocial behavior, annual cycle of colony population, distribution of tasks among castes of bees in the colony and foraging behavior. Honeybees cannot be treated as other test organisms because they have the most complex social order. The honeybee colony has been called a ‘superorganism’. No individual bee can survive and reproduce outside the colony and colony survival and growth depends on the collective actions of different castes and a single queen. Even the queen can be replaced by the actions of worker bees. Reproduction is also done at the colony level by the process of swarming, which is controlled by many factors beyond the health of the queen. Contrary to normal colony behavior, which allows only a single queen to live in the hive, a swarm-bound colony produces multiple queens, and up to 70% of the population leaves the hive along with the original queen. The remaining workers and a virgin queen are vulnerable to attack by other honeybees, or by other pests and diseases and may not succeed in rebuilding. For the beekeeper, swarming is a major cause of colony loss. (7) The conflicting protection goals are discussed further below but the key consideration for risk assessment is that the unit of replication for honeybee risk assessment is the colony. (4)

The regulation of the annual cycle of colony population responds to many natural factors and is also influenced by management practices. In temperate regions, a typical bee colony builds up rapidly after overwintering to make foragers available to take advantage of short-lived food supplies as various floral sources come into bloom. The summer population peaks at approximately 70,000 bees under honey production conditions in North America, although colonies maintained for pollination are restricted to a smaller size. But there are too many bees to sustain after the flowers are gone. Significant food stores are often used to survive between the summer and fall flowering periods; this is a time when robbing becomes a serious threat to survival. In response, egg-laying slows and since the typical life span of these summer worker bees is less than 38 days, the population drops. None of the summer bees except the queen remain to form the winter cluster, which is made up of roughly 10,000 workers that have a life-span that may exceed 140 days. In the following spring these winter bees survive until the first cohorts of summer bees emerge as adults. (8). This gives rise to a very rapid turnover of bees in the colony and large changes in population. Furthermore. large shifts in population may occur during normal beekeeping operations. In honey production, the majority of foragers in a colony are displaced when honey is taken off, particularly late in the season when the colony is reduced to one or two brood boxes for winter. It is apparent that unless the colony is already at a critically low population, (e.g. after a swarm) there is a large (10-30%) redundancy of worker bees. This shows that the honeybee colony is very resilient to large changes in population.

For honeybees, the third major consideration in bee biology is the distribution of tasks among castes of bees in the colony. Worker bees progress through a loosely organized series of task groups, although not all of them do all tasks. Rather, groups of worker bees are recruited to various tasks as needed.(7) Newly emerged bees clean the hive and cap cells, then progress to caring for the brood and queen, followed by comb building, grooming and food handling. The oldest workers undertake guard duty and foraging, and continue to forage until they die. These are the only bees with activities outside the hive. Thus, the foragers are the most expendable bees in the colony. In this intricate social structure the forager bees are also the most directly exposed to toxic substances in the environment i.e primary exposure. Except in extreme incidents like direct overspray contrary to product label instructions, all other task groups of workers are exposed only to residues brought to the hive by foragers (secondary exposure). or by off target movement in air.

Different castes and task groups of bees have different potential exposure in terms of duration, magnitude, and route. It is essential to take these differences into consideration for even the lowest tiers of risk assessment and to include both the routes of exposure and the relevant efficiencies or transfer factors for each route. Foragers collect much more pollen, nectar and water than for their own needs; they have higher potential exposure. Nurse bees consume much more food than for their own nutrition, and have higher potential oral exposure.

With competition for food resources, under variable climate and under different disease and pest stress levels, and many factors such as queen replacement that are a matter of probability it is apparent that colony growth and development will be highly variable and difficult to replicate.

Finally, it has been said that the large forage area of honey bees complicates the task of estimating and avoiding potential exposure, but for risk assessment it is important to note that honeybees often focus on 1-3 main food sources and a worst-case single source is reasonable.

2.3 The Quantitative Pollinator Conceptual Model

The goal of the conceptual model is to guide the calculation of the aggregate exposure for each caste and life stage of bee. This is required to determine the ratio of potential exposure to a measure of the toxicity of the material being assessed (the Risk Quotient). Also, while many toxicology tests consider a single standard duration of time (e.g. 96 h), under actual conditions of exposure, the dose arrives and dissipates over a time scale that may be much shorter, resulting in greatly reduced toxicity. In the determination of the risk, the duration of time for the toxicity end point must match the time interval of exposure. The QPCM goes beyond other conceptual models by considering the distribution of the material after application into the various compartments in in the environment and in the bee colony where exposure occurs and the dissipation or depurition of residues from the bee and from the colony.

The potential exposure for various life stages of other pollinators such as bumblebees and solitary bees can also be considered within this model either as a subset of the same exposure routes or with inclusion of several additional routes peculiar to these species.

3. Discussion

3.1 Description of the Model

The detailed QPCM conceptual model was constructed to satisfy the requirements described above. The exposure scenario is considered as a network of compartments represented by rectangles and flow pathways shown as arrows from the point of application of the pesticide or stressor to the point of action or receptor (top left and right of Figure 1). Three of four major phases of exposure are shown in Figure 1: distribution among environmental compartments where exposure may occur; primary exposure of the individual pollinator and secondary movement and exposure of other individuals. E.g., the transfer of food to other bees, whether offspring or other adults, may result in secondary exposure. The fourth phase, shown in Figure 2, includes the pathways for dissipation. For all compartments and bees there is a kinetic pattern of increase, transformation and decline in concentration with time.

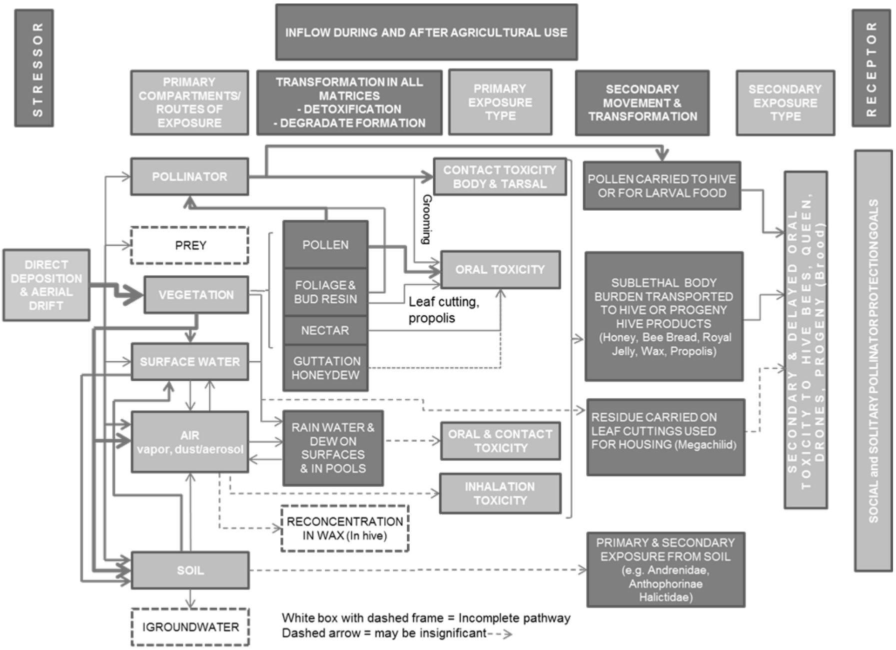

Figure 1 Conceptual Model for Pollinator Exposure Inputs

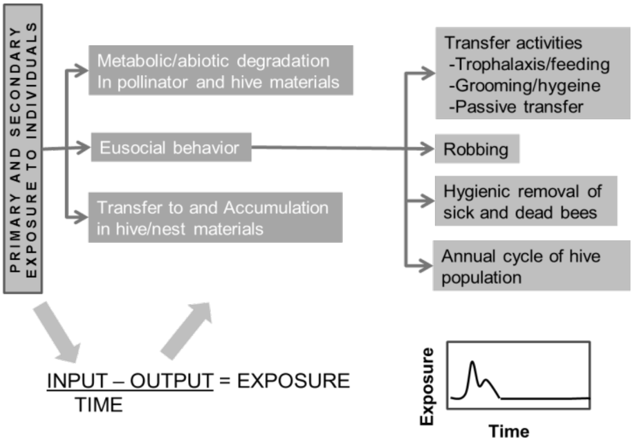

Figure 2 Conceptual Model - Dispersal From The Receptor Organism or Colony

Some pathways that might apply in a general sense are not applicable or incomplete for the case of honeybees and other bees. For example, exposure via consumption of prey. These are compartments with a dashed-line border. Possible but insignificant pathways are indicated by a dashed arrow flow line, while important pathways are shown by increasingly broad lines. The distribution of material after application depends on the mode of application, e.g. spray vs seed treatment, but for a spray application as illustrated, material will initially be deposited on exposed plant surfaces, soil and any water that may be present on the surfaces. Offsite movement may result in exposure to non-target plants and soil but at much reduced levels. Individual insects may receive a direct external dose via spray or dust if they are in the area of direct spray or drift. After application there are multiple processes that redistribute the material among the various compartments, and alter the bioavailability of the applied material. These can be quantitatively modeled in response to weather conditions. The potential exposure in each environmental compartment can be considered separately.

Vegetation is divided into subcompartments that relate to the foraging behavior of the individual honeybee or non-*Apis* bee: these are pollen, nectar, foliage and bud resin, and guttation/honeydew. Residues may partition into water on wet foliage, on the ground or in pools, whether from rain or dew. This water is considered a separate exposure compartment to allow for a different potential rate of transfer of residues to the pollinator. Pollen collection and contact with foliage may add to the externally carried dose on the pollinator, while the nectar, guttation water, honeydew and orally consumed pollen add to the oral dose. Surface water contributes to both oral and contact toxicity, and airborne vapours contribute to an inhalation dose. With the exception of pollen, these contribute to an aggregate body burden that represents the primary exposure. A portion of the pollen-borne residues may be carried to the nest and transferred without the carrier being exposed. While these pathways represent mainly the flow of residues for the highly eusocial honeybee, several pathways are included to represent non-*Apis* bees. These include oral exposure of leaf cutting bees to residues on foliage, and exposure of ground nesting bees or mason bees to residues on soil. Secondary exposures are also represented for the offspring of these species. For honeybees, the transfer of residues among individuals within the colony occurs via both consumption of food stores by individual and by trophallaxis, but transfer to larvae and reproductive castes is tightly restricted as these individuals are fed metabolic secretions (royal jelly) by nurse bees (Purdy). Each of these primary and secondary exposures has an efficiency or transfer factor.

The pathways and rates of decline of residues shown in Figure 2 are also an essential component of the overall conceptual model. The detailed kinetic balance of increase and decrease in body burden with time is what determines the effect on the individual bee and the sum of these effects on individuals is what governs the outcome for the colony or for a population of solitary bees.

3.2 Cofactors

A vital component of pollinator risk assessment that is often overlooked is the importance of major influences on colony health that change rapidly with time and are difficult to control.(1) The colony typically survives in a delicate balance between growth supported by resources and decline due to the many endemic stressors they face. Nutrition may be a limiting factor since some major pollen sources contain protein that lacks amino acids necessary for larval development. Diseases, predators and parasites, particularly *Varroa* have been repeatedly identified as the main causes of bee colony losses around the world. (9-11) According to Bailey and Ball "Viruses have probably always been prime sources of confusion and error in the diagnosis and management of bee diseases". (12) This is of particular significance now, since virus and other disease symptoms are being promoted as neonicotinoid toxicity effects._ENREF_13 (13),(14) Given that it is not possible to do research on bees in the absence of these factors, bee studies must include or control health, nutrition, beecare and other cofactors.

3.3 Significance

Preliminary indications of the success of this model-based risk assessment approach are available from consideration of several published reports. The work of De Grandi Hoffman et al on chlorpyrifos showed that there was a decline in exposure of more than one thousand fold from the primary exposure of adult foragers to chlorpyrifos in almond pollen through the secondary exposure of hive bees and nurse bees to the royal jelly fed to the queen and young larvae.(5) In work with a series of neonicotinoids (Purdy 2014, published herewith), primary exposure concentrations up to 14.7 ppb in pollen, ppb in 8.2 ppb in nectar/honey and 2.4 ppb in forager bees were found, but there were no detectable residues in hive bees (nurse bees) and hence no exposure to the queen or young larvae. The detections were limited to during and after planting in

May and June. These results demonstrate the need to report exposure of foragers separately from exposure to hive bees and reproductive castes and also to separate the risk assessments.

3.4 Protection Goals

It is also possible to consider the significance of the conceptual risk assessment model in terms of the protection goals. Difficult contradictions may be seen among protection goals that have been established. For example, a queen with a high level of fecundity will build a colony up fast enough to trigger swarming. Swarming is natural reproductive success at the colony level but is detrimental for the protection goals of pollination services and hive products in the context of commercial beekeeping and detrimental to survival of the parent colony in general. Uncontrolled reproduction contributes to species abundance but is simply a cost and a source of infestation and disease from feral colonies to the beekeeper. High rates of growth occur in strong healthy colonies under above normal warm spring conditions and the resulting early swarms are seen as spring colony losses.

4. Conclusions

- Eusocial behavior is a major determining factor in the honeybee risk assessment
- The unit of replication in honeybee risk assessment is the whole bee colony
- The bee colony is resilient to loss of large numbers of workers or drones, and can even replace the queen
- Cofactors of bee health, nutrition, beecare etc must be considered
- The conceptual model describes the flow pathways and potential exposure routes for honeybees and other bee pollinators in sufficient detail to support quantitative exposure modelling and risk assessment. The model may be adapted for other pollinator species _ENREF_6 (6)

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