Risk assessment to honey bees: a scheme developed in France for non-sprayed systemic compounds

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Abstract

BACKGROUND: Directive 91/414/EEC envisages that the systemic properties of active substances, if any, are taken into account in evaluating the risk posed to the environment by plant protection products. Among others, honey bees may be exposed to substances via this route, which may pose problems when substances with high toxicity are ingested through pollen or nectar. The guidance documents in support of the risk assessment to bees within the framework of Directive 91/414/EEC do not provide detailed technical guidance on how to proceed in a risk assessment for substances with systemic properties.

RESULTS: A stepwise approach aiming specifically to assess the risk posed by non-sprayed systemic substances to bees is therefore proposed. This approach first identifies substances with systemic properties, which should be quantified in plant material as pollen and nectar. Exposure estimates calculated for different categories of bees (e.g. foraging bees), based on expected concentrations of the product in pollen or nectar, may be compared with several toxicity endpoints for acute or chronic effects on adults and/or larvae with a toxicity/exposure ratio, which is a measurement of potential risks.

CONCLUSION: Such a ratio is proposed to be used as a trigger for any further refined assessment that would focus on the measurement of effects at the colony level.

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Keywords: risk assessment; honey bees; systemic compounds

1 INTRODUCTION

According to Council Directive 91/414/EEC, the placing on the market of plant protection products relies on the prior demonstration that these products, under the proposed conditions of use, pose no unacceptable risk to human health or to the environment. The risk to bees is evaluated within this framework, with the aim of protecting honey bees and other pollinating insects. The risk to bees is evaluated according to a common approach for all the groups of organisms addressed by Directive 91/414/EEC, which recommends the following course of action: (i) explore exposure conditions; (ii) if exposure cannot be excluded, investigate possible effects and determine ecotoxicity endpoints; (iii) quantify exposure of organisms under the proposed conditions of use of the plant protection product; (iv) evaluate the risk, in most cases by comparing the ecotoxicity endpoints with the exposure level.

As far as bees are concerned, they are considered to be exposed except in the case of indoor uses such as in food storage in enclosed spaces and in glasshouses without pollinators. Other uses with low expected consequences to bees are included, such as wound sealing and healing treatments, rodenticidal baits and outdoor uses as non-sprayed treatments provided that the products are non-systemic, such as non-systemic seed dressings, non-systemic preparations for application to soil and non-systemic dipping treatments for transplanted crops and bulbs. When exposure may occur, effects are investigated, firstly by laboratory tests exposing honey bees through either the oral or the contact route and determining acute LD$_{50}$ values for both administration routes. Exposure is quantified as the application rate which may vary depending on the use. The risk is then assessed by calculating the following hazard quotients (HQs) for oral and contact risks:

\[ HQ_{\text{contact}} = \frac{\text{application rate}}{\text{LD}_{50}\text{ contact}} \]
\[ HQ_{\text{oral}} = \frac{\text{application rate}}{\text{LD}_{50}\text{ oral}} \]

with the application rate expressed in the same substance as the ecotoxicity endpoint (g active substance (a.s.) or formulated product (f.p.) ha$^{-1}$), and the LD$_{50}$ expressed in µg a.s. or f.p. bee$^{-1}$. The HQ is then a non-dimensional quotient that somehow represents the number of LD$_{50}$ in one

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treated hectare. The quotient is compared with a trigger value of 50, as recommended in the decision-making criteria of Directive 91/414/EEC. If both HQs are below that trigger value, then the information is considered sufficient to exclude any further impact of the product on bee populations under the conditions of use evaluated. If one of the HQs is above that trigger value, then additional information is required further to investigate the toxicity pattern (residual toxicity to adults, sublethal effects, toxicity to larvae) and determine the nature and extent of the impact on the colony. This further investigation may include residue tests and cage, tunnel and field tests in an attempt to reproduce more realistic exposure conditions of bees. The risk assessment scheme thus enumerates four steps easily adapted to most products used outdoors.

In practice, this risk assessment scheme has proved to be adequate for the evaluation of the risk posed by the products being sprayed onto plants, but it appears to be of limited relevance for non-sprayed products, as it was not designed to address these. For systemic products, the relevance of the application rate is questionable as an exposure estimate, the impact assessment as an adult bee ecotoxicity endpoint, and no trigger value is validated for the resulting HQ. Recent regulatory reviews of plant protection products pertaining to systemic pesticides have required a huge amount of information generated through dedicated studies in order to quantify impact and exposure. New data are produced on a case-by-case basis, which is at the edge of the general harmonisation philosophy of Directive 91/414/EEC, and makes the extrapolation of evaluation tasks between Member States difficult.

This paper analyses the risk assessment scheme as currently proposed in Directive 91/414/EEC and related guidance documents on the specific question of non-sprayed products. The specificity of the exposure and impact components of a risk assessment approach related to non-sprayed products is discussed in light of a literature review. The dedicated risk assessment approach, as discussed in France, for non-sprayed plant protection products is presented, together with an exploration of the issues required to improve and adapt a risk assessment scheme to bees to more specific modes of application of plant protection products in the field.

2 CURRENT RISK ASSESSMENT SCHEME FOR NON-SPRAYED PRODUCTS

2.1 Directive 91/414/EEC

Directive 91/414/EEC addresses the question of non-sprayed products very succinctly. Two annex points detail the uses for which the exposure of bees cannot be excluded, namely systemic seed dressings, systemic preparations for application to soil and systemic dipping treatments for transplanted crops and bulbs. Then, decision-making criteria (annex VI of Directive 91/414/EEC) mention, among the information to be used in the evaluation of the risk to bees, ‘where relevant, any information on the persistence of residues in the treated plants’. The decision is made on a common basis, since protection aims at all substances, whatever the mode of application, by considering that ‘where there is a possibility of bees being exposed, no authorisation shall be granted if the hazard quotients for oral or contact exposure of honey bees are greater than 50, unless it is clearly established through an appropriate risk assessment that under field conditions there are no unacceptable effects on honey bee larvae, honey bee behaviour, or colony survival and development after use of the plant protection product according to the proposed conditions of use’.

2.2 Guidance documents in support of Directive 91/414/EEC

Guidance documents provide few additional recommendations on the exposure statement and on the triggering for toxicity tests. The mode of application, defined as soil treatment involving a systemic plant protection product, triggers an acute oral toxicity test with the active substance. For substances for which a risk is identified at this stage (e.g. very low LD50), it is proposed to ‘take into account realistic exposure conditions, for example, exposure concentrations as expected in nectar and pollen as indicated by residue studies’. However, no other indication is provided to trigger this step, although it is recommended that exposure, with which the oral LD50 could as an example be commonly compared, should be ‘expressed based on the compound (active substance or metabolite) present in the respective plant parts (e.g. nectar, pollen) to which honey bees could be exposed’. The next step, triggered by a risk at this stage, would be to envisage ‘higher-tier studies (cage/tent/tunnel or field studies) with realistic exposure scenarios’. In fact, documents quite quickly return to suggesting higher-tier studies to address potential risk, mainly because ‘estimates of the concentrations of compounds in the relevant plant parts are rarely available’ and ‘exposure calculations in higher-tier studies are already considered within the experimental design (e.g. honey bees foraging on treated field crops)’.

Finally, the critical HQ of 50 was validated against field studies with sprayed products and is then only applicable to sprayed products (Oomen PA, private communication). There are no validated decision-making criteria applicable to a first-step risk assessment for non-sprayed compounds. This risk assessment scheme has been drawn as a diagram in Fig. 1. In this scheme, systemic properties are confirmed as a prerequisite that trigger further risk assessment for non-sprayed products. However, no definition is provided concerning the elements to consider in establishing the active substance as ‘systemic’ (e.g. residues in plants demonstrated in what type of tests, etc.). Secondly, the possibility of risks is identified on the basis of available information, which is in principle a single acute oral toxicity test on adults. Since the HQ calculation has no relevance

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for non-sprayed products, and because the scheme requires residue quantification data in bee food at a further step (which are rarely available), the potential for a risk is evaluated on the basis of the acute oral LD₅₀ values for adults. Criteria for a classification of substances within toxicity classes, based on LD₅₀ values and the slope of the dose–response curve, have already been proposed but again are dependent on observations of effects in field studies following a spray use rather than systemic use of the products. At the next step, the difficulty in detecting and further quantifying residues in the relevant part of plants is highlighted. If this can be solved, then impact assessment by undertaking tunnel/field studies may allow exposure of the honey bees to be established analytically. The last main gap relates to the triggering criteria to progress in this stepwise approach and to the decision-making criteria for granting authorisation.

3 SPECIFICITY OF BEE EXPOSURE TO NON-SPRAYED PRODUCTS

3.1 Exposure modalities

Exposure of pollinating insects to pesticide residues differs greatly depending on the mode of application of products. Exposure to sprayed products occurs through contact with surface residues and, at blossom, through the collection of contaminated plant material (nectar and pollen), in which case uptake may occur by both contact and oral routes. Residual action is generally rapid, occurring over several days or even hours, pesticide residues potentially dissipating through volatilisation, leaching, or photodegradation. Oral action is also expected to be of short duration, and may occur through cleaning or through the ingestion of residues from pollen or nectar after treatment at blossom, in which case exposure duration is the same as for residual action. In the case of preblossom treatments with a systemic product, it may contaminate pollen or nectar from within the plant and thus may expose insects for a longer period. In that case residue dissipation is dependent on plant metabolism rather than volatilisation, leaching or photodegradation, and it may be expected that, if the metabolism rate is slow enough for residues to be present in blossoms, then further degradation may also not be very rapid. Concern relating to exposure to non-sprayed products mainly relates to systemic products, and it occurs through the collection and consumption of plant materials such as nectar and

Figure 1. Risk assessment scheme for non-sprayed products as deduced from Directive 91/414/EEC and guidance documents Sanco/10329/2002 and EPPO.
pollen. That means that the exposure of insects in the case of a soil or seed applied systemic product is mainly a chronic and oral issue, while the exposure of insects in the case of a systemic product being sprayed is for the most part a short-term and contact issue, since exposure to the largest fraction of the dose applied occurs around the spray event, and exposure to the systemic part depends on factors such as blossom time relative to treatment.

Honeydew from sap-sucking insects, such as aphids, is also attractive to bees, and exposure is expected when the honeydew is contaminated by direct spraying or from inside the plant if the substance is systemic. In this case another condition for exposure is that the substance remains stable in the honeydew-producing insects. Depending on the plant species, significant honeydew amounts may or may not be expected within the range of good agricultural practices. This issue has then to be considered in the light of knowledge about plant attractiveness under normal cropping conditions.

3.2 Presence of honey bees in treated fields
A key factor that affects the exposure of pollinating populations to non-sprayed products is attractiveness of the cropped plant constituents to them. If the cropped plant is not attractive to pollinating insects, exposure may then occur only occasionally and result in non-significant consequences at the population level. Some crops are regularly visited by honey bees, such as oil seed crops, orchards or vegetable crops, whereas other crops are only occasionally visited, such as vine or cereals in the case of food shortages. Various lists of plants of interest to pollinating insects exist in the literature, but none of them is definitive or agreed for the purpose of risk assessment within Directive 91/414/EEC.

The question of the attractiveness of crops should not be limited to the plant species being ‘protected’ (i.e. being treated). It may also be considered in a longer timescale that is defined by the fate of the product applied in the field. Slowly dissipating substances may, if they remain in significant amounts in the soil after harvest, be mobilised by the following crop, the attractiveness of which becomes a key question for the risk assessment. The occurrence of translocations of soil residues in succeeding crops is commonly assessed in the European review process and mainly relies on field data.

3.3 Systemic properties and contamination of nectar and pollen
For non-sprayed products, the systemic property of the active ingredient is then, besides the attractiveness of the crop, an entry point of the risk assessment scheme. Examples exist of nectar and pollen contaminated by systemic substances being sprayed on crops even when blossoms were covered during the spray. Other studies demonstrate the translocation of residues in nectar after soil treatment with granules. However, very few studies have focused on the analysis of nectar or pollen for pesticide residues after soil or seed treatments, although available results indicate the presence of quantifiable amounts of residues that may in some cases result in the need to proceed to a risk assessment.

A substance is referred to as systemic, with respect to plants, if it is taken up, primarily by plant roots, and transported to locations throughout the plant. Systemic residues move within the vascular tissues, either through the xylem or the phloem, depending on their characteristics. In the context of pesticides, substances are referred to as systemic on the basis of the results achieved in residue studies. These studies are conducted on crops treated at the intended rate with the aim of checking for the presence of any residues, including the active substance and its byproducts, and, when relevant to quantifying them, in edible parts of the plants. The residues analysed at crop stages close to the flowering period would be the most relevant for the present purpose, but these stages are not always included in the sampling procedure of studies primarily designed for the determination of a maximal residue limit (MRL).

With respect to the resulting risk to bees and insects in general, however, clear pathways of exposure are difficult to establish and quantify. The presence of residue in pollen or nectar is rarely assessed in routine studies. The presence of residues in green parts of plants does not necessarily imply the presence of residues in pollen or nectar. Studies measuring residue concentrations in nectar or pollen indicate that the amounts of residues reaching these matrices may correspond to variable fractions of the dose applied, depending on the plant species and environmental conditions. Translocation of pesticides is specified to be measurably less effective to fruiting structures than to other plant parts. Studies comparing residue levels among plant parts, and particularly between green or edible parts and nectar or pollen, are sparse. Translocation of herbicides for log values of 4 and above. Mobility in phloem translocation of herbicides has been analysed together with lipophilic properties as informed by the 1-octanol/water partition coefficient (log $P_{ow}$) and with dissociation properties as informed by their pK_a. The analysis concluded that there was a negligible translocation of herbicides for log $P_{ow}$ values of 4 and above. Mobility in phloem was found to be satisfactorily predicted by log $P_{ow}$ for the ca 400 substances analysed, with pK_a modulating the log $P_{ow}$ influence in extreme values.
3.4 Quantifying the exposure of honey bees

The exposure of honey bees involves both contact and oral routes in adults and immature stages, although the routes may not have the same importance in the two age groups. Nectar and pollen foragers are the most directly exposed to non-sprayed pesticides, as they collect plant material to bring it back to the colony. Hive bees are exposed to the collected material, i.e. nectar and pollen, and the brood is exposed to both raw and transformed, i.e. honey and royal jelly, material. Contact of larvae with pesticide residues may occur through contact events with adults, during the release of eggs in the cells, during feeding and cell inspection or through contact with the wax of cells, as shown for particular substances such as organochlorines. However, except in the latter case, exposure through contact between adults and brood remains occasional. As a consequence, the oral route may be considered the major one in the case of larvae.

Evidence of pesticide transfer from plant product to larvae has been demonstrated in studies using radio-labelled insecticides, found to accumulate slowly in the rectum. The risk of transfer from nectar to larvae depends on the stability of the substance during honey formation (e.g. sensitivity to action of enzymes such as invertase) and thereafter in the honey sac. Various types of insecticide were found to be stable in honey, which may relate to the absence of mixed-function oxidase enzymes in the honey sac. The concentration of residues in the honey to which larvae are exposed also depends on the level of condensation during the formation of the honey.

The level of contamination in nectar and pollen may correspond to various levels of exposure depending on the age and role of bees within the colony. As an example, the amount of sugar, brought as nectar, that is consumed by adult honey bees is a function of their activity and energy needs. Food consumption in the honey bee has recently been reviewed and is summarised in Table 1. Food consumption is expressed, for bees differing in development stage and status, in mg sugar or mg pollen bee$^{-1}$, for a period defined for each bee category (5–90 days). Data also include larvae, as a transfer of substances through honey and royal jelly proved to be measurable for some systemic substances. Figures for larvae corroborate values proposed for risk assessment by other authors. Details of calculations and literature references can be found in the review by Rortais et al.

4 NATURE OF EFFECTS IN EXPOSED BEES AND TESTING

The collection by foragers of syrup containing a lethal quantity of insecticides has been experimentally observed, but investigation in the field of the impacts of plant systemic pesticides on honey bees has been limited and primarily focused on sprayed products.

Both foragers and hive bees may be exposed if contaminated plant material is brought back to the

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Differences and the resulting toxicity endpoint is available for now easily determined through a standardised test, and the acute oral toxicity of the residues to adults is residues, result in sublethal or lethal symptoms. Products may, depending on the toxicity of the colony, and effects on one of the bee categories (age and status) may impact on the colony at different levels. The nature of these impacts is briefly discussed below in relation to the consequences at the population level.

### 4.1 Adults

Adults consuming contaminated food and hive products may, depending on the toxicity of the residues, result in sublethal or lethal symptoms. The acute oral toxicity of the residues to adults is now easily determined through a standardised test, and the resulting toxicity endpoint is available for active substances in public databases. Differences in sensitivity to acute effects among adults have been observed, e.g. nurse bees being more sensitive than foragers to insecticides. A relatively low specific activity of two detoxifying enzyme systems in nurse bees was proposed as an explanation.

More insidious are sublethal effects in adults, as they are less easy to detect in the field, while they may affect the colony at a significant level. In a study coupling a monitoring of colonies exposed to spiked food with a honey bee population model, fenoxycarb was found to be associated with observed failure in mating for the queens, which also failed to produce eggs, resulting in reduced winter size of 40–50%, and so affecting the ability of the colony to overwinter. Pyrethroid insecticides were also associated with decreased foraging in glasshouse and field studies where the products were applied at the recommended application rates. Other behavioural impairments, as summarised in Table 2, may also compromise colony survival and strength in the long term.

Insecticides, and also fungicides and herbicides, have been observed to induce behavioural impairments in adult honey bees, at least under laboratory conditions. Owing to the lack of field studies and the difficulties in monitoring most behavioural parameters in these studies, it is often not possible to quantify the occurrence of such effects under realistic exposure conditions and their meaning for the survival of the colony. It is expected, however, that effects on parameters directly related to the development of the colony, such as reproductive behaviour, ability to requeen and queen fecundity, may be more damaging to colony vitality than effects on foragers. Behavioural symptoms may occur in each stage of honey bee adulthood and in all categories of bees, with examples of substances involved in impairments at several stages. In most cases these effects have been recorded in bees exposed to realistic dosages of substances, or to dosages below the level of residues currently encountered in the field.

### 4.2 Larvae

The intrinsic acute toxicity of pesticides to brood has been far less investigated than for adults, both from a regulatory point of view and in the research area. To date there is no equivalent guideline for the testing of acute effects on larvae to those existing for adult bees. As a consequence, very little information is available to propose any statement on the relative sensitivity of larvae compared with adults. Available data comparing the acute toxicity of various pesticides to larvae and adults revealed an important variability. Of the 31 substances tested as technical-grade or formulated product, three were less toxic to larvae than to adults (toxicity was considered different when LD50 values were higher or lower by at least an order of magnitude), 21 were equally toxic and six were more toxic to larvae. No comparison could be made for one substance. Differences reached a factor ranging from 3 to 100. No conclusion could be drawn on the predictability of the toxicity to larvae from the chemical family or the mode of action of the substance tested, the highest differences (e.g. ratio of LD50 > 100) being observed for both ‘simple poisons’ (diazinon, profenophos) and insect growth regulators (chlorfluazuron). For substances showing a higher toxicity to larvae, the ratio of LD50 ranged from 30 (oxamyl) to >200 000 (chlorfluazuron), the latter being non-toxic to adults (LD50 > 100 µg bee\(^{-1}\), limit test). The sensitivity of larvae may evolve during the development, with the LD50 value increasing, decreasing or remaining stable with ageing (larvae of 1–2, 3–4 and 5–6 days were tested).

The available information on the sublethal effects of pesticides on honey bee larvae mainly concerns former

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**Table 1. Consumption of pollen and sugar in the different honey bee categories, adapted from Rortais et al. (2005)**

<table>
<thead>
<tr>
<th>Categories of bees</th>
<th>Sugar (mg)</th>
<th>Pollen (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Workers (5 days)</td>
<td>59.4</td>
<td>5.4</td>
</tr>
<tr>
<td>Drones (6.5 days)</td>
<td>98.2</td>
<td>n.a.</td>
</tr>
<tr>
<td>Hive bees</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nurses (10 days)</td>
<td>–</td>
<td>65</td>
</tr>
<tr>
<td>Wax-producing bees (6 days)</td>
<td>108</td>
<td>–</td>
</tr>
<tr>
<td>Brood-attending bees (8 days)</td>
<td>272–400</td>
<td>–</td>
</tr>
<tr>
<td>Winter bees (90 days)</td>
<td>792</td>
<td>–</td>
</tr>
<tr>
<td>Foraging bees</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nectar foragers (7 days)</td>
<td>224–898.8</td>
<td>–</td>
</tr>
<tr>
<td>Pollen foragers (7 days)</td>
<td>72.8–109.2</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^a\) n.a. = data not available.
Table 2. Behavioural effects of pesticides observed on the honey bee, their occurrence under realistic exposure conditions in the field and observed or expected impact on the colony, from the review of Thompson (2003)\textsuperscript{32}

<table>
<thead>
<tr>
<th>Type of effect</th>
<th>Family of substance or mode of action</th>
<th>Expected or observed effect on the colony$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division of labour</td>
<td>Juvenile hormone analogues, organophosphates, carbamates, pyrethroids, neonicotinoids</td>
<td>Expected because of consequences on lifespan and colony leading to foraging. Unclear as far as dances are concerned (organophosphates, carbamates) as bees are likely to seek out forage.</td>
</tr>
<tr>
<td>Foraging</td>
<td>Organophosphates</td>
<td>Expected in the case of low return to the hive associated with further recruitment of nurses.</td>
</tr>
<tr>
<td></td>
<td>Carbamates</td>
<td>Observed under field conditions for pyrethroids.</td>
</tr>
<tr>
<td></td>
<td>Pyrethroids</td>
<td>Observed for increased time for habituation and learning.</td>
</tr>
<tr>
<td></td>
<td>Neonicotinoids</td>
<td>Not observed in field studies for imidacloprid.</td>
</tr>
<tr>
<td>Conditioned responses</td>
<td>Pyrethroids</td>
<td>Expected because it directly acts on the colony renewal.</td>
</tr>
<tr>
<td></td>
<td>Neonicotinoids</td>
<td>Expected because it directly acts on the colony renewal.</td>
</tr>
<tr>
<td>Reproductive behaviour</td>
<td>All classes</td>
<td>Expected because it directly acts on the colony renewal.</td>
</tr>
<tr>
<td>Comb production</td>
<td>Dimethoate</td>
<td>Expected because it directly acts on the colony renewal.</td>
</tr>
<tr>
<td>Failure to requeen</td>
<td>Organophosphates, pyrethroids, neonicotinoids</td>
<td>Expected because it directly acts on the colony renewal.</td>
</tr>
<tr>
<td>Queen ability to lay egg</td>
<td>Pyrethroids, neonicotinoids</td>
<td>Expected because it directly acts on the colony renewal.</td>
</tr>
<tr>
<td>Nestmate recognition</td>
<td>Herbicides</td>
<td>Expected if it leads to mortality in worker bees.</td>
</tr>
<tr>
<td></td>
<td>Fungicides</td>
<td>Reported by beekeepers of the UK National Bee Unit.</td>
</tr>
<tr>
<td>Repellency</td>
<td>Pyrethroids, azadirachtin, fipronil, captan</td>
<td>Expected if there are losses of foragers and further recruitment of nurses.</td>
</tr>
<tr>
<td></td>
<td>Herbicides</td>
<td>Observed in the field for pyrethroids.</td>
</tr>
<tr>
<td></td>
<td>Neonicotinoids</td>
<td>Not observed for azadirachtin and fipronil.</td>
</tr>
</tbody>
</table>

$^a$ Absence of reference to field results means that no result was published.

Table 3. Effects of pesticides in honey bee larvae and observed or expected impact on the colony (detailed references may be found in reference papers\textsuperscript{27,34})

<table>
<thead>
<tr>
<th>Type of effect</th>
<th>Family of substance or mode of action</th>
<th>Expected or observed effect on the colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low hatchability of eggs</td>
<td>Juvenile hormone analogues, chitin synthesis inhibitors, IGR</td>
<td>Expected because it directly acts on the colony renewal.</td>
</tr>
<tr>
<td>Easily ruptured larvae</td>
<td>Juvenile hormone analogues, chitin synthesis inhibitors, IGR</td>
<td>Expected because it directly acts on the colony renewal.</td>
</tr>
<tr>
<td>Abnormalities in post-larval stages</td>
<td>Juvenile hormone analogues, chitin synthesis inhibitors, IGR</td>
<td>Depends on the handicap raised by the abnormality.</td>
</tr>
<tr>
<td>Abnormal size</td>
<td>Organophosphates</td>
<td>Depends on the amplitude of the effect.</td>
</tr>
<tr>
<td>Abnormal tanning</td>
<td>Organophosphates</td>
<td>Expected since it is associated with failure to pupate.</td>
</tr>
<tr>
<td>Unsuccessful larval and prepupal moults</td>
<td>Organophosphates, azadirachtin</td>
<td>Expected because it directly acts on the colony renewal.</td>
</tr>
<tr>
<td>Amorphogenesis</td>
<td>Juvenile hormone analogues, azadirachtin</td>
<td>Expected because it directly acts on the colony renewal.</td>
</tr>
<tr>
<td>Antifeeding behaviour</td>
<td>Organophosphates</td>
<td>Expected because it directly acts on the colony renewal.</td>
</tr>
</tbody>
</table>

generations of substances, as very few studies have been published more recently that would describe such effects for the more recent substances. Examples of such effects have been reported and are summarised in Table 3.\textsuperscript{26,33} The main difficulty in assessing sublethal effects in adults is that the observations would have to be performed inside the colony, which presents technical constraints. As a consequence, very few field studies manage to associate overall effects on the colony at study termination with the impairment of a particular step in the development of larvae. Effects on a particular development stage are not necessarily related to a particular chemical family or mode of action, as, for example, organophosphates may act both on the feeding capacity and on moulting, and some of these symptoms may actually be linked. The relevance of these effects to the survival of the colony depends on the amplitude of the effects but also on the status of the stage in terms of developmental success. As an example, hatchability failure has a more direct

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and definite impact on development than a reduced weight because the larva is still alive in the latter case and may successfully complete its development.\textsuperscript{33}

Developmental effects in larvae may also have consequences for the behaviour of emerged adults and therefore affect colony survival.\textsuperscript{31} Dimethoate, malathion, carbaryl and the fungicide captan have been reported to induce morphogenic effects in adults exposed as larvae, such as small-sized adults, wing malformations, in some cases wingless, stunted bodies, crippled legs and wings.\textsuperscript{31,32} These effects, observed at realistic levels of exposure, may severely affect the ability of the adults to perform duties within the colony.\textsuperscript{31}

5 FACTS FOR A RISK ASSESSMENT SCHEME

5.1 Triggering a risk assessment by establishing exposure

Considering that authorisations are granted on a crop/use basis, information on the attractiveness of the crop to pollinators is required for the risk assessment. An inventory of plant species being attractive to pollinators could be drawn up, including the plant species being visited by honey bees for honey production, but also the species that need the visit of pollinators for reproduction. To address this data gap, information may be gathered from the literature, but a general and agreed view is required.

As stated above, the fate of the active substance and of its residues in the soil–plant system has to be considered in exploring the potential exposure of pollinators following the use of a product, and information may also be required on the attractiveness of succeeding crops. Both fate endpoints such as DT\textsubscript{50} or DT\textsubscript{90} (the disappearance time for 50 and 90% of the substance) of the relevant residues in soils and results from residue studies in succeeding crops, the two types of data being routinely generated in the regulatory context,\textsuperscript{1} are useful at this step. Residue studies in succeeding crops are generated for high values of the fate descriptors (DT\textsubscript{50} and DT\textsubscript{90}) and investigate translocation in green and edible parts of plants. The difficulty in extrapolating residue levels in blossoms, nectar or pollen from the residue levels measured in these studies has been discussed above, particularly with regard to the risk of either over- or underestimating exposure. In that context, as these studies are performed in all cases for systemic and persistent products, they could be extended with additional residue analysis in blossoms and even nectar and pollen, in order adequately to establish and quantify exposure of honey bees to the relevant residues.

One important aspect at this step is that the limit of quantification to be used in analysing residues in those parts of the plants may need to be readjusted when necessary, e.g. in the case of active substances inducing effects at very low concentrations. Similar adjustments for quantification limits in surface water are performed for aquatic organisms.\textsuperscript{1} This means that, for very toxic substances for either adults or larvae, the exposure is established on the basis of analytical methods being adapted to the toxicity level, which relies on cross-references between analytical and ecotoxicological data.

5.2 Investigating effects through a tiered approach

5.2.1 Effects on adults

In adults, acute oral toxicity is measured following a single exposure event, after which effects are checked over a 48h observation period.\textsuperscript{2,3} Corresponding standardised tests have been developed in order to determine the intrinsic toxicity of the xenobiotic to adults, which are then useful to position the relative toxicity of a substance, to be compared with that of other substances, formulated products and even metabolites, and even to discuss the adequacy of the limit of quantification to be used to quantify exposure in plant material. These tests, however, may be of less relevance in describing the potential lethal impact of repeated or chronic consumption of contaminated food. As for birds,\textsuperscript{7} a prolonged feeding test may be more representative of the exposure pattern to systemic products being translocated and remaining stable in honey bee food. A method for measuring the toxic level of orally administered substances to caged bees over a 10 day period has been published.\textsuperscript{34} Such a method would bring a suitable endpoint for chronic and repeated exposure of foraging bees [LD\textsubscript{50} for survival or preferably NOEL (No Observed Effect Level) for survival and sublethal effects] but requires further validation to be routinely used in a regulatory context.

As far as sublethal effects in adults are concerned, the OECD\textsuperscript{2,3} and EPPO\textsuperscript{4} guidelines require all abnormal behavioural effects to be recorded, but there is no additional guidance about the types of effect to be assessed or about the experimental design to measure them. These effects are neither taken into account in the risk assessment as the endpoint for effects from 48h acute tests is the LD\textsubscript{50}, nor as NOEL for all the observed effects.\textsuperscript{1,3} Some sublethal effects may be observed in a 10 day prolonged toxicity test and included in the endpoint to be used in the risk assessment. Several laboratory methods have been developed in order to measure effects on parameters such as the learning potential, locomotion, orientation ability or communication, but the relative part that these parameters play in colony development and vitality has not been documented sufficiently for the results of these tests to be used alone in the risk assessment.\textsuperscript{31,35,36} Such tests may complement, when necessary, cage, tunnel and field tests that include observations of foraging activity and behaviour but do not integrate other observations.\textsuperscript{4} Some of the behavioural impairments listed in Table 2 are almost impossible to follow outside the hive, so that they cannot be investigated in
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5.2.2 Effects on brood
Initially, laboratory tests for oral and contact toxicity were recommended on the life stages most at risk, which, as the risk assessment scheme focused mainly on sprayed products, included adults and more particularly foragers\(^1,7\) (Oomen PA, private communication). The increased recourse to application techniques different from spraying, including soil treatment and seed dressing, together with the improvement of the efficacy of insecticides and pesticides in general, may justify investigation of the ecotoxicity of these products to all the life stages actually exposed. This is most important, as available data indicate no basis for extrapolation of the acute toxicity from adults to larvae\(^32\) using, for example, an extrapolation factor. The requirement for a test with larvae in establishing the effects of pesticides on honey bee colonies has already been recommended, based on observations on the developmental success of larvae orally exposed to contaminated food.\(^33,34\) Such a recommendation is supported by the extreme difficulty in predicting the occurrence of larval impairments from the chemical family to which a substance belongs, and from the acute oral toxicity of the substance to adults. This is also supported by the difficulty in further predicting the consequences that these impairments represent on the developmental success. Such tests should be performed in cases where the substance remains stable enough in the plant materials being collected, is resistant to the action of invertase in the honey bee stomach and remains stable enough thereafter to lead to a chronic and repeated exposure. Test methods have been developed for investigating the oral toxicity to the brood of free-flying colonies\(^39,40\) that expose bees through either a feeder delivering contaminated syrup\(^30\) or through treated flowers grown in the tunnel.\(^40\) These tests are well adapted to provide integrated responses about the impact of the tested product on the colony, provided that the exposure of larvae is controlled, which remains difficult without disturbing the bees. The measure of the intrinsic toxicity to larvae, which requires a laboratory test at the scale of individuals and thus would be equivalent to laboratory testing methods for adults,\(^2,3\) needs the current rearing methods to be improved and standardised.\(^41\) Such a test, measuring lethal effects (both LC\(_{50}\) and LD\(_{50}\)) and developmental success in larvae (through both NOEC and NOEL) whose exposure would be controlled and quantified\(^41\) (no interaction with nurses, control of food delivery and consumption), would constitute a necessary preliminary to further colony-scale tests.

5.3 Stepwise risk assessment scheme and decision-making criteria
For spray-applied products, entering the risk assessment is triggered as soon as an outdoor application is intended. Then, high-tier tests (cage, tunnel and field tests) are triggered by a former risk assessment step consisting of an HQ calculation\(^7,8\) (Oomen PA, private communication).

In the case of non-sprayed products, entering the risk assessment is triggered when exposure of pollinators is expected, i.e. for systemic products being translocated in attractive crops (Fig. 2). This step relies on data being in part already available in European dossiers, including systemic properties of the substance in the protected crop species, persistence of the relevant residues in soils and related residue studies in succeeding crop, and on knowledge about the attractiveness of those plants to pollinators. A definite statement about exposure is more adequately assessed on the basis of the residue concentrations in nectar or pollen than on the basis of residue concentrations in the edible part of the crop.

This step would in principle trigger a first investigation of the toxicological effects of the substance and relevant byproducts under laboratory conditions, in order to distinguish substances being of clear concern to pollinators from those that pose no particular risk to pollinating species. For this purpose, a new risk assessment calculation should be implemented, in the form of either a toxicity exposure ratio (TER)\(^21\) or a PEC/PNEC\(^35\) ratio, both constructed to integrate exposure as concentrations (µg residue g\(^−1\) or kg\(^−1\) food) or as estimated exposure (µg residue g\(^−1\) or mg\(^−1\) body weight). In France, the PEC/PNEC ratio is preferred, as it already integrates the assessment factor, if any.\(^35\) Transformation of data from concentration to ingested doses may be performed by multiplying residue concentration in food with the food requirement of the different bees of the colony (Table 1), as has been done for imidacloprid.\(^6\)

Considering that such a ratio would be used to trigger a higher-tier test, it would include acute or chronic ecotoxicity endpoints measured in laboratory tests, and expected or measured levels of exposure in plant material. In that case, the inclusion of an assessment factor in the PNEC calculation has to be envisaged, as extrapolation from laboratory to field,
and possibly also from acute to chronic toxicity. In France, no definite assessment factors have been determined, the risk assessment scheme having not been used with many products to date.

Such an initial risk assessment step to trigger the requirement for a higher-tier test is particularly important in the case of non-sprayed products. The main reason for this is that extrapolations of the results achieved under realistic exposure conditions from one crop to another are rarely relevant. This is due to the fact that the exposure of honey bees relies on the application rate, the nature of the cultivated species and the properties of the active substance. For two plant species with a similar application rate, the amount of active substance or byproducts reaching the blossoms and nectar or pollen relies on systemic properties in both plants and on further metabolism. At this stage, the exposure of bees relies on the relative attractiveness of the two plant species, so that exposure is not directly predictable on the basis of the application rate. When the decision-making is performed on a dose and crop basis, the extrapolation limits then inevitably lead to manifold high-tier study requirements in order to proceed to a satisfactory risk assessment.

6 CONCLUSIONS

The application methods for plant protection products in crops is evolving towards methods that reduce losses and related contamination and improve the fraction of the dose applied that reaches its target. In that context, there is a need for risk assessment approaches to be adapted to application methods such as soil treatment, topical seed treatment or plant dipping, in which efficacy relies on behavioural properties of substances in the whole soil–plant (and insect) system.

The risk assessment scheme dedicated to the honey bee and to pollinating insects does not follow this philosophy, and a detailed approach for non-sprayed application methods is lacking at the European level. Preliminary work is needed to: (i) describe and

predict the processes leading to pollen and nectar contamination; (ii) develop or improve analytical methods dedicated to residue analysis in nectar, pollen and relevant materials; (iii) develop relevant routine laboratory tests for assessing the effects of substances on the relevant steps of the life cycle of bees; (iv) develop methods and models able to predict the impact on the colony from effects on these steps.

Systemic compounds are of particular concern within the context of non-sprayed products, but most of them can also be sprayed onto crops. In this case, however, the risk assessment will fail to separate the effects due to direct or residual contact from the effects due to the systemic behaviour of the compound, and delayed effects following applications before the flowering period are often the only signs of a systemic action on non-target insects. Links between the two approaches need to be revisited on the basis of the experiences gained for non-sprayed compound risk assessment.

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