Risk assessment of pesticides on bees: evaluating risk coefficients for assessing acute and chronic toxicity

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Abstract

Background: Risk coefficients are the key in the way how a pesticide active substances or formulated products will go through the risk assessment scheme dichotomy. Defining them is thus one of the main challenges of a risk assessment scheme design. In the light of the scientific publications on the subject, the existing risk coefficients and methodologies used for the toxicity evaluation under international guidelines result questionable.

Results: LD50 values have shown to be variable. Prolonged effects following single contact can sometimes be observed when measuring the acute toxicity. The trigger value (10) of the risk coefficient results as inadequate. The toxicity derived from the exposure to substances continuously available for bees at sub-lethal doses needs to be evaluated separately, given the wide differences between acute and chronic lethal effects of pesticides.

Conclusions: The observation period of the mortality tests should be lengthened as long as mortality increases, and while control mortality remains acceptable. Whenever active substances can pollute bees' food sources, first tier tests should include laboratory tests: (1) on adult bees with: (a) acute toxicity tests; (b) chronic toxicity tests; (c) behavioural tests; and (2) larvae toxicity tests. Consequently, the decision to run higher tier tests should depend on four different risk coefficients.

Keywords: honeybees, risk assessment, acute toxicity, chronic toxicity, risk coefficient, TER

Introduction

The EPPO risk assessment scheme of pesticides on bees has recently being updated.[1] The aim was to include the evaluation of pesticides with systemic properties. In parallel, the legal framework[2] currently demands any active substance, safener or synergist to have negligible exposure to honeybees and not to show unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour under the proposed conditions of use of the product containing it.

The scheme proposed starts with a screening of the potential acute toxicity of the product under evaluation to adult bees. In case the substance can be problematic for larvae, the toxicity on brood will also be evaluated. An IGR or active substances showing toxicity to larval stages by screening or efficacy studies will be tested for brood effects. On the basis of the data obtained from this first phase, data on the LD50 of the active substance in adult bees is produced (contact and oral). When contrasting these values to the exposure potential, active substances might be further evaluated for their chronic toxicity or being classified as low risk for bees based on the comparison with a trigger value of a risk coefficient. The exposure is determined from the concentration of the pesticide in the aerial parts of the plant, considered an overestimation of the residues found in pollen or nectar (default value 1 mg/Kg).

Several publications have put in question the role of certain systemic active substances in the problem of bee decline. The observed effects range from a direct or indirect shortening of bees lifespan (leading in the long term to the collapse of the colony),[3-5] the disruption of the reproductive capacity of queen and drones,[6,7] the synergistic effect with pathologies[8,9] or the cases of acute intoxications following seeding operations.[10-12] The capacity of the EPPO scheme to discriminate between active substances that might be problematic to bees, especially in the long run, has been tested with the existing ones. Precisely, this risk assessment scheme has been calibrated to fit the

characteristics of the majority of the active substances put into question. Mainly those active substances with toxicity values in the range of ng/bee would require further testing (unless residue levels are in the range of mg/Kg). However, the capacity of the scheme to determine the impact of chronic exposure, or if other existing or future active substances could have potential acute or chronic effects on colony survival and development[2], remains uncertain.

The present article proposes a non-comprehensive analysis of the toxicity variables included into the definition of the risk coefficient categorising the risk of active substances. In order to verify if the system proposed fits the legal requirements, data extracted from a literature review were used for the analysis of the scheme.

Results and Discussion

The current risk assessment dichotomy of EPPO relies on the value of the Toxicity Exposure Ratio (TER) based on the comparison of the LD50 and the exposure (in terms of bee consumption per day). Should the TER be lower than 10, chronic toxicity studies would be run. Otherwise, the active substance would be characterised as low risk for bees.

1. Variability of LD50 and extrapolation to real conditions

It is worthwhile analysing the parameters conforming the TER. Despite the proposed standardised methods for its estimation, LD50 varies widely. The LD50 of imidacloprid, for example, has shown values between 3.7 and 40.9 ng/bee,[13] 40 and 60 ng/bee,[14] 49 and 102 ng/bee[15] and 490 ng/bee[16]. Some sources of variability are colony genetics or bee management during testing. Data have shown the variation in the measured LD50, considering different parameters or variables like temperature,[17] the age of the bees,[17,18] the bee sub-species,[19] the pattern of exposure (unique vs multiple exposure),[20,21] the exposure of the tested bees to a pesticide prior to acute testing,[21] etc. Given the diversity of the parameters mentioned in real field conditions and the different exposure to pesticides of the individuals of the colony, Belzunces in 2006,[21] suggested that LD50 values should only be used as a comparison tool among pesticides. However, this value alone should not be used to draw conclusions about the level of risk to bees in the environment.

2. Prolonged effects

OECD guidelines[22] currently recommend the daily recording of mortality at least up to 48 hours. Should the mortality rate increase between 24 and 48 hours, while control mortality remains acceptable (≤10%), an extension of the duration of the test should be to 96 hours. Certain active substances have shown increased mortality over this observation period. Suchail *et al.,* 2001,[14] showed prolonged action of imidacloprid and two of its metabolites (olefin and 5-hydroxy-imidaclopred) up to 96 hours, some of these substances showing an tendency to increase. The same is shown for fipronil sulfone, the oxidative metabolite of fipronil.[23] The toxicity evolution beyond this observation period is not known. These effects might be the result of the long-term residual effectiveness of the mother compound or the bio-activation of toxic metabolites. A longer observation period as long as there is an increase in toxicity could be envisaged, as long as the control mortality would not rise above unacceptable values. Such a modification of the methodology for the determination of LD50 would involve minimal changes in test management, but provide precious information about the toxicity kinetics of the active substances and products under evaluation. It could be argued that the existence of prolonged effects could be evaluated through tunnel tests. However, these tests are not systematically run. Furthermore, prolonged effects may result from an extended exposure to the pesticide in the tunnel.

3. Exposure - PEC

Different exposure patterns and durations can be expected depending on the use and properties of pesticide products or the behaviour and function of each of the colony members. Furthermore, a bee colony may be repeatedly exposed to the same substance in different ways at different periods of the year. Bees can get in contact with systemic active substances: (1) spread in dust (following seeding

operation of certain treated seeds)[11,24] or in the air (following spray applications); (2) in plant exudates[25-27]or superficial water;[28] (3) in pollen and nectar;[29-31] (4) present in the reserves of the colony.[32-34]

Toxic molecules suddenly distributed in the air (following spraying or seeding operation of some treated seeds) might affect foragers in an acute way. The contamination of nesting material might entail a risk to the colony members from inside the hive. The contamination of food and water sources involves, depending on the doses, either acute or chronic exposure to pollutants. The long persistence of the active substance in the environment increases the risk of chronic exposure. Indeed, residues in pollen, nectar or honey of systemic compounds can range from 0,7 µg/Kg (imidacloprid)[35] up to 94 mg/Kg (carbaryl).[36] These figures are worrying considering that honeybees should negligibly be exposed to active substances.[2]

Considering the wide range of pesticides residues found in food matrixes, the default value of 1 mg/Kg may be inadequate. A more precise approach would be the analysis of residues directly in the matrixes following the treatment.

4. Risk coefficient - TER

The trigger value established by the EPPO guidelines for the risk coefficient (10) "[…] aims at ensuring a margin of safety that is sufficient to cover the uncertainty related to longer exposure periods and possible related increased effects […]". Ideally, this safety value should cover as well the uncertainty related to the appearance of sub-lethal effects that could impact the colony and the uncertainty related to the capacity for extrapolation of the scheme to field conditions (in case such tests would not be undergone).

The value of 10 is based on unpublished results of a DEFRA study[16] showing a potential 10-fold adjustment factor between LD50 (µg/bee) and LC50 (µg/bee/day). Chronic toxicity is observed after 10 days of continuous exposure to pesticides in lab conditions.[1] The consequence of this value could be very important. Supposing the acute (oral) LD50 of a substance is 5 ng/bee, then the estimation of the chronic LC50 would be 0,5 ng/bee. Following the proposed principle and assuming a bees' exposure of 0,49 ng/bee, the TER calculation would be larger than 10 (5/0,49). Exposure to 0,49 ng/bee could be observed in numerous active substances already found in residue studies.[29-31,35] Consequently, an active substance or product would be categorized as low risk to bees, even though the bees' exposure would be almost equal to the chronic LC50.

A literature review has been carried out identifying studies done to determine the chronic toxicity of pesticides (after continuous exposure over more than 10 days).[37-41,14,16] Bearing in mind their differences in experimental set-ups, the same ratio has been calculated in order to have a notion of the magnitude of the safety factor that could be necessary. LD50/LC50 values of 31 active substances show a range from 0,51 (acetamiprid)[16] to 100.000 (imidacloprid metabolites).[14] If instead of continuous exposure, repeated exposure is considered (intermittent doses, 17 active substances), the ratio show a range from 0,05 (for emamectin)[42] to > 1.000 (various active substances).[20,42] A parallel exercise could be done with a comparison of LD50 values with doses showing sub-lethal effects without leading to mortality in the long run.

Therefore, several active substances have shown to be lethal when administered to bees at concentrations lower than those inducing acute mortality in case they are administered over a long period. The hypothesis provided to explain the differences in mortality between acute and chronic exposure are based on toxicity dynamics and kinetics: (1) existence of high and low affinity receptors, low doses activating high-affinity receptors (inducing mortality through an agonistic effect) while high doses would activate both low and high-affinity receptors (proving a compensation action);[14] (2) enzymatic bio-activation of mother compounds into toxic metabolites;[14,43] (3) detoxification capacity of bees exceeded by the daily intake of the toxic material.[41,42,44] All in all, it seems that there is no clear correlation between acute and chronic toxicity.

As a result, it seems inappropriate to use a risk coefficient (TER) based on acute terms (LD50 and an overestimation of the exposure of bees to the contaminant) to determine if chronic toxicity tests need to be run. A more suitable proposal would be, first to check the pattern of exposure of the

different colony members - exposure over short/long periods may happen. Depending on this result, either acute toxicity tests or both acute and chronic toxicity tests in adult bees should be carried out as first tier tests. These oral tests are simple, not cost intensive and provide good information about the toxicity of the active substance. Risk coefficients that would use, respectively, acute and chronic toxicity values, together with the respective exposure (to residues in spray, dust, food sources, etc.) would determine the necessity of running higher tier tests.

The trigger value of the risk coefficients using both acute and chronic terms should include a safety or uncertainty margin that would consider the variation of the toxicity parameter used.

5. Other necessary first tier tests

It needs to be noted that these toxicity tests and risk coefficients would refer only to the potential lethal effects on adult bees. Regulation (EC) 1107/2009 specifically mentions that an active substance, safener or synergist should not have unacceptable effects on honeybee larvae and honeybee behaviour under the proposed conditions of use. Brood effects should be considered separately and systematically in case pesticide exposure cannot be avoided through contamination of food sources. Therefore, toxicity testing on larvae should not be based just on the mode of action of the active substance or on screening or efficacy studies. A third risk coefficient including parameters targeting brood (toxicity and exposure) should be included into the scheme to determine if higher tier studies are needed.

Similarly, several techniques evaluating the appearance of sub-lethal effects on adult bees at very low doses are currently available. Considering the importance of behaviour and communication in social insects, it should be envisaged to include in the first tier the potential impact on bees of these sub-lethal doses. Realistic low doses of pesticides found already in bee colonies' food and water sources should be used for the tests. This is in accordance with the recommendations provided by Tasei et al., 2003.[45] The laboratory tests proposed so far (for example Proboscis Extension Reflex test) are not complicated to carry out and would complete the evaluation of pesticide active substances and products. Again, a risk coefficient including sub-lethal parameters confronted to a trigger value would determine if further studies are requested. The results of these studies may help, as well defining the observation parameters to focus while running higher tier tests.

Furthermore, the observation and systematic recording of behavioural and locomotion effects happening during mortality tests could complement these specific behavioural or developmental tests. Nevertheless, a clear and standardised scale of effects should be defined.

Conclusions

The present article argues why the EPPO risk assessment scheme is not satisfying for the evaluation of pesticides with systemic properties. As a result, several improvement proposals have been presented. The first one aims to achieving a better methodology to determine the LD50 by increasing the duration of the observation period until the mortality is stable as long as control mortality remains under acceptable levels. The second one shows the importance of reviewing the trigger values of the risk coefficients used in risk assessment. The third one presents the need to run chronic toxicity tests systematically in case food and water sources of bees can get contaminated with the active substance under evaluation. Acute and chronic exposure to contaminants entails differences in toxic dynamics and kinetics. Therefore, chronic toxicity tests should be carried out independently of the results of acute toxicity tests at first tier test in the risk assessment. Similarly, larvae toxicity tests and specific tests evaluating the impact of sublethal doses of pesticides should be included in first tier in case food sources can become contaminated. As a result, four different risk coefficients would determine if higher tier tests are needed: (1) acute toxicity/relevant exposure (oral or contact); (2) chronic toxicity/relevant exposure (oral); (3) larvae toxicity/relevant exposure (oral); (4) dose producing sublethal effects/relevant exposure (oral). Trigger values will need to be defined based on present and future studies and certitude assessment.

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