II. Test and risk assessment (incl. systemic effects, field testing, bee brood)

Risk Assessment of Pesticides and the role of EFSA

Karin M. Nienstedt

EFSA, Largo N. Palli 5a, Parma, Italy

Abstract

The European Food Safety Authority (EFSA) was created by the Regulation EC 178/2002 on 28 January 2002 with the mandate to provide scientific advice and support for the European Community policies in all fields with impact on food and feed safety. The PPR Unit (Plant Protection Products and their Residues Unit, Risk Assessment Directorate) as well as the Pesticides/PRAPeR Unit (Scientific Cooperation and Assistance Directorate) both works on Plant Protection Products in relation to Directive 91/414 EEC. PRAPeR coordinates the Pesticide Risk Assessment Peer Review for the approval of active substances by the European Commission and the Members States, whereas the PPR Panel provides independent scientific opinions and guidance for the Community's legislation in the field of plant protection products.

Actual examples have been presented regarding the role, working procedures and results of the PPR Panel and PRAPeR in relation to the risk assessment of plant protection products to bees (e.g. EFSA-Opinions, EFSA-Conclusions). Information on on-going and scheduled work of the PPR Panel in this area have also been mentioned. In line with EFSAs commitment for transparency, details of the ongoing work are published on www.efsa.europa.eu.

Systemic plant protection substances and products: how to assess the risk for bees? A beekeepers point of view

Etienne Bruneau[1], Janine Kievits[2], José-Anne Lortsch[3] , Szaniszlo Szöke[4]

[1]Centre apicole de recherche et d'information, 4 place Croix du Sud, 1348 Louvain-la-Neuve, Belgium
0032 (0)10 47 34 16 (bruneau@cari.be)
[2]Inter-Environnement Wallonie, 6 boulevard du Nord, B-5000 Namur, Belgium - 00 32 (0) 81 25 52 80
[3]Syndicat national d'apiculture, 5 rue de Copenhague, F-75008 Paris, France
[4]Agronomist and beekeeper, 16 rue de Brionsart, B – 5340 Gesves, Belgium

Abstract

Background: The current plant protection products (PPPs) assessment is no more suitable when applied to systemic substances since systemic chemicals can contaminate nectar and pollen during a long length of time. Largely focused on the acute toxicity, the current assessment scheme does not take into account several elements i.e. the chronic toxicity, the possible synergies between substances, and between pathogens and PPPs. Possible bee contamination through nectar and pollen leads to a specific exposure, mainly oral, concerning the hive bees, including larvae, drones and queens, as well as potentially delayed through the stored honey and pollen consumption. Moreover, regarding the long-term exposure, sublethal chronic effects should be taken into account.

Results: For such substances we would take both the chronic toxicity and the acute toxicity measurements into consideration. Therefore the TER should be calculated based on the lowest LD_{50} and in the case of risk, the PEC/PNEC ratio should be measured and calculated for various behaviours. A larvae test should also be performed. Tunnel tests may be helpful but the exposure to the PPP cannot be proven and the bee behaviour observation is currently inaccurate. Further research on the effect of small doses of PPP on the bee immune system seems more than necessary.

Conclusion: A new assessment scheme, which takes these parameters into account, is the core of our contribution.

Keywords: Assessment scheme, chronic toxicity, sublethal toxicity, synergies, larvae test, PEC, PNEC, TER.

Introduction

Like all other pollinators, honeybees are gathering everyday thousands of micro-samples from their environment. Because of their wide foraging area and their intense foraging activity, they are already used as bio-indicators (Porrini et al. 2003).[1]

Moreover, the honeybee has fewer genes encoding detoxifying enzymes than other arthropods. Therefore, bees seem to have less capability of detoxification than most of the other species of insects (Claudianos et al. 2006).[2]

The bee colony, as a super-organism, can survive only if key-pheromone relations and numerous complex behaviours are preserved.

Depending on the flowering, the bees perform sophisticated foraging strategies, which can vary from year to year, influenced by the temperature and the rainfall conditions. Food is stored for long periods and the consumption of harvested nectar and pollen can therefore be delayed of several months. Finally, the colony is composed of different classes and castes of bees and the toxicity of a single substance therefore varies between classes and casts.

These three facts demonstrate by themselves the importance and the difficulty to perform an accurate assessment of the PPPs.

The current plant protection products assessment does not satisfy us entirely, especially when applied to systemic substances. Systemic chemicals can contaminate nectar and pollen, during the entire blossom. Largely focused on the acute toxicity, the current assessment scheme does not take into account several elements such as the chronic toxicity, the toxicity variation between bee classes and bees castes, the possible synergies between substances and the possible synergies between pathogens and PPPs.

The objective of this document is to show alternatives to the definition of an assessment scheme, in order to consider the specificity of systemic substances and PPP particularly when they are suspected to contaminate pollen and nectar.

Specificity of the bees exposure to systemic PPPs

Opposite to sprayed non-systemic products, the systemic products, particularly when used as seed or soil treatment, lead to a different and specific bee exposure. This specific exposure is described in some scientific publications (e.g. Alix and Vergnet, 2007).[3]

To be very clear in this exposure we would remember the main conditions:

- Honeybee is exposed through their feed sources, nectar and pollen, and not through the drift when flying. It would appear to be mainly an oral exposure (opposed to sprayed PPPs leading mainly to a contact exposure).
- The contaminated food is brought back to the hive where it will be used by the whole colony. The food can contaminate all casts: workers, drones and queens and all the other classes: nurses, storekeepers, foraging and winter bees.
- The nectar and the pollen brought to the beehive will be stored. The nectar can be used immediately. On the contrary the pollen requires a one-week fermentation to be digestible by the bee. In addition, the stored food will be consumed during the periods of the year outside harvest time and particularly during wintertime. Thus a pollen collected in August may be consumed the following March or even early April; the consumption being delayed by up to 8 months.

- Considering the flowering timescale and the possible storage contamination, which will later be consumed, the actual contamination timeframe can be extended to long periods (opposite to sprayed products which are generally quickly downgraded by photolysis). Possible contaminations can thus have chronic lethal and sublethal effects. If the PPP is a neurotoxic, the sublethal effects may concern any behavioural pattern.

We add to the above that some of the concerned PPPs are already found in significant concentrations in the environment (Chauzat et al. 2006).[4]

A new assessment scheme

Given the specificity of the honeybee's exposure to systemic substances when present in the foraged matrices, it is necessary to develop a new assessment scheme. We argue it will not be relevant to adapt the current scheme for many reasons:

- The "trigger value" (Hazard Quotient =HQ > 50) is not entirely relevant.
- The current higher tier tests are not sufficient to assess effects.

The current higher tier tests are not sufficiently reliable since the chronic effect assessment needs long-term effects assays.

The trigger value

Relevance of the HQ for systemic PPPs used in seed soil treatment

The HQ coefficient is only validated for products used in sprays (SanCo 10329, p 18).[5] The HQ takes into account the acute, oral and contact LD_{50} only, as well as the application rate. The persistence and the chronic toxicity, which are both essential parameters to appreciate the risk level of systemic PPPs are not taken into account.

Villa et al. (2000)[6] propose a method for assessing the risk of contaminated pollen via a TER (Toxicity Exposure Ratio) calculation based on physical and chemical properties (P_{oa}[1]), persistence, application rates and LD_{50}. This study concludes that the comparison between the two approaches (TER and HQ) shows a relatively good result but points out that chemicals with a high $logK_{oa}$ are classified as more dangerous by TERs in comparison to HQ.

Even for a sprayed product, the HQ reliability seems to be low when the substance is systemic.

We can make the conclusion that the systemicity must be estimated at the first tier assessment, both for sprayed products and for products used as seed or soil treatment.

Obviously this does not mean that a high HQ matches with a small risk for honeybees when the product is systemic. A high HQ should always be a warning about the PPP risk for bees because it indicates that the spread amount is high compared to the acute toxicity.

Elements to consider at the first tier step

Every evaluation of PPP risk for honeybees should start considering the following points:

- The acute LD_{50} and the HQ
- The octanol/water (octanol/air) partition coefficient as systemicity indicator
- The persistence
- The presence of the substances and their metabolites in the foraged matrix, pollen and nectar. This presence must be detected by using analytical methods, which have the lowest limits of detection and quantification. Especially, these limits must be of the same order of magnitude as the toxicity of the substances on bees.

- The application type for the active substance: some substances used in soil or seed treatment are designed to protect the whole plant during its growth (e.g. insecticides in seed treatment). This kind of PPPs should always be considered as systemic substance

When the substance is considered as a systemic and persistent[2] product and /or when the PPP is used as seed treatment (to protect the whole plant), the trigger value should be a TER.

When the products and substances are present in the foraged matrix, the chronic toxicity (both lethal and sublethal) should be assessed; the TER should take into account the lethal chronic toxicity and, except when the TER shows a low risk, the sublethal effects are to be assessed.

For the sublethal effects assessment, the PEC/PNEC approach (predicted environmental concentration / predicted no effect concentration ratio) seems to be the only appropriated way today (Halm et al. 2006).[7]

Then the products and substances will be directed to a particular scheme as soon as the systemicity is attested.

Proposal for the first tier assessment

We would propose the following scheme:

First tier assessment: acute LD_{50} + systemicity

A substance is systemic:

- if (log P_{ow}, log P_{oa},) + persistence leads to a risk index > X
- if detected in the foraged matrices
- if applied as seed or soil treatment and aimed to protect the whole plant

Higher tier assessment

When low systemicity:

- and HQ < 50: no higher tier test

and HQ > 50: higher tier tests of current assessment scheme (cfr EPPO 170)[8] (bee brood feeding test, cage tests, tunnel tests, field tests).

Higher tier assessment

When low systemicity:

- and HQ < 50: no higher tier test
- and HQ > 50: higher tier tests of current assessment scheme (cfr EPPO 170)[8] (bee brood feeding test, cage tests, tunnel tests, field tests).

Higher tier assessment

When low systemicity:

- and HQ < 50: no higher tier test
- and HQ > 50: higher tier tests of current assessment scheme (cfr EPPO 170)[8] (bee brood feeding test, cage tests, tunnel tests, field tests).

---

[1] P_{oa}: partition coefficient octanol/air. The log P_{oa} is directly proportional to the uptake by leaves of hydrophobic organic chemicals from the air. This study is concerned with sprayed PPPs absorbed by the plants leaves as vapour.

[2] for instance: log Pow < 5 et DT50 > 7days; log Pow is given to PH = 4, PH=10 and the smallest value is considered.

When the substance is considered systemic: chronic LD50 + bee exposure => TER calculation when the TER shows a low risk:

- and HQ < 50: no higher tier tests
- and HQ > 50: higher tier test of the current assessment scheme

When the TER shows a risk: bee brood feeding test and PEC/PNEC assessment.

TER calculation

TER = ratio between the LD_{50} and bee exposure (consumed substance quantities). The LD_{50}s considered are oral and contact, acute and chronic. The quantity of the consumed substance by the bee depends on:

- the substance concentration in the pollen and the nectar (measurements)
- the amount of pollen or nectar actually consumed by the bee (estimation)

For each TER, the quantity of the substance considered is:

- the amount consumed by the bee in real conditions, as it can be evaluated (CST report)[9] (Rortais et al. 2005)[10]
- the amount consumed within the considered LD_{50} timeframe (48H or 10 days)

When the bees have consumed both nectar and pollen of the considered plant, the amount to be taken into consideration should be the sum (amount consumed through the pollen + amount consumed through the nectar).

Acute toxicity

The acute toxicity is assessed following the current rules.

If the relation dose/mortality curve shows irregularities or variations at the trial replications, the safety factor should be greater than usual (TER_2>TER_1, cfr scheme below).

Chronic toxicity

The trial design is similar to the acute toxicity determination, except that the total dose of substance is divided into ten daily doses (from day 1 until day 10) given in the morning. If the relation dose/mortality curve shows irregularities or variations at the trial replications, the safety factor should be greater than the usually used one (TER_2>TER_1, cfr scheme below).

Chronic toxicity / acute toxicity ratio

Calculating this ratio is a way for assessing the potential accumulation of a substance. The chronic toxicity test cannot last more than 11 days in laboratory since the bees do not bear confinement. When the ratio between acute and chronic LD_{50} is greater than 2, the sensitivity to repetitive doses is more than twice the sensitivity to a single dose, meaning that a clear cumulative effect is observed. In such case, a greater safety factor should be used for the TER calculation.

Concentration measures in the foraged matrix

The analytic methods used should permit the concentration detection at a comparable level to the NOEC or LOEC considered during a long period of time; however the NOEC and the LOEC are not yet determined at this step of the assessment.

We would propose a limit of quantification LoQ ≤ LD_{50}/200.

For instance, a product for which the LD_{50} = 5ng/bee - that is to say 50ng/g of bee -, the limit of quantification must be at most 0,25ng/g (0,25 ppb). Such quantification limits are nowadays possible; methods are described, for instance by Bonmatin et al. (2006)[11] (LoQ : 1 ppb ; LoD : 0,1 ppb).

For the pollen, the current analytic method must include the dissolution or the grinding of the pollen envelopes because the toxic substances are located inside the grain and not on its surface.

We would emphasize too that it is not relevant to validate an analytic method by testing its ability to detect the substance when spread onto the pollen.

The pollen should come from pollen traps or better, from the flowers because this is the one consumed by the bee. The trap pollen needs to be sorted out when the studied contamination is linked to a specific crop. The comb pollen is usually a mix of different pollen sources, which conduct to contamination dilution. This fact should be taken into account when sampling and for the forthcoming test conclusions.

Quantity determination of pollen/nectar consumed by the honeybee

The quantities of pollen and nectar considered for the exposure estimate are of course the amounts consumed by the bee in real conditions. Many publications and reports estimate food quantities consumed by a bee or a colony. Concerning the pollen, 65mg is given for a nurse upon 10 days (Rortais et al. 2005)[10] or also 160 to 180mg for a worker during its whole life (Keller et al. undated).[12] Pollen amounts consumed by winter bees are unknown at this time. Every beekeeper knows that wintering may only succeed if the bee colony has collected important quantities of pollen during summer. Most of this pollen will disappear during winter and early spring: it has been consumed by the bees, and particularly by nurses for feeding the early brood. The winter bees are not numerous (10 000 – 15 000) and they will feed brood for a long period. This means that pollen consumption per winter bee is potentially more important compared to summer bees. Thus the pollen toxicity for winter bees should then be tested specifically. Before carrying out this test, it is necessary to quantify the pollen amounts consumed by winter bees with great care in order to define their exposure.

Concerning the nectar, Rortais et al.(2006)[10] quotes a range of 72,8 (wax foragers) to 898,8mg/bee (nectar foragers). Beekeepers can make a quick estimation: a colony harvests 60 kg of honey, that is to say 150 kg nectar during one month time (a usual average amount during the sunflower blossom). Then, two forager generations must be considered, or about 20 000 foragers (it is commonly considered that a hive contains about 10 000 foragers simultaneously). During its lifetime, each honeybee will harvest 7.5 g of nectar from which about 10 % is used for the forager itself. So each bee will consume about 750 mg in 2 weeks, or 107 mg in 48 hours. The main part of the nectar is not immediately consumed; it is brought back to the hive, and regurgitated to be stored into the combs, or shared with other bees of the colony. This part can generate a contact toxicity: it is not digested, however it is in contact with the oesophagus and the stomach of the honeybees. This contact toxicity is never taken into account in the current TER assessment. The consumed quantities taken into account should be pursuant with the principle of the *worse case* (harvested amounts in important honey-supplier crops as sunflowers e.g.).

Proposal for the trigger value

The trigger value takes into account a safety coefficient, which should cover the sublethal effects. This coefficient should vary according to 3 parameters:

- the steadiness of the results from replications
- the regularity of the mortalities curves
- the cumulative effect from doses (ratio between the acute and chronic LD_{50}).

A bad reproducibility of the results, or irregular mortality curves show uncertainties that should be covered by a greater safety coefficient; and sublethal effects appearance is more likely when this substance gets accumulated into the bee body.

The PEC/PNEC approach

The sublethal effects from the chemical ingredients on the bee behaviour or other useful insects are reported by a great number of publications (Desneux et al. 2007)[13]. For instance, sublethal doses of insecticides impair the waggling dance (parathion), the harvest and the transport of the nectar (diazon), the homing flight (deltamethrin) (Vandame et al. 1995)[14].

Regarding the honeybee, we find in the scientific literature remarks about:

- development
- survival, fertility and egg-laying capacity of the queen
- the mobility of the bee
- the bee capability to find its way on short distances (using the visual or olfactory memory)
- the bee capability to find its way on long distances (using the aptitude to find its way according to the position of the sun and the memory associated to that capacity)
- the behaviour when feeding and the training capacity
- foraging intensity
- thermoregulation

Moreover PPPs are likely to reduce the honeybee length of life (essential parameter for the harvest), its immune capacity, and other behaviours that are necessary to the integrity of the colony and its natural development, such as:

- the bee brood feeding
- the whole behaviour leading to swarming
- the combs construction and the balance between drone cells and worker cells
- the search for a new nest in the swarming period and the transmission of information to the other pioneer bees ….

A complete bee behaviour model does not exist today; we do not believe that such a model could be possible considering the behavioural complexity of this super-organism.

The question has sometimes been asked about the ecological relevance/reliability of the sub-lethal tests for the concerned effects on behaviour. Despite the fact that man has grown bees for a long time, their physical and behavioural characteristics have not been altered. All aspects of the behaviour likely to be affected have a utility in the survival and good development of the colony. We have no knowledge of any scientific element that would blame or deny this postulate.

The sub-lethal effects can vary according to several parameters

- The sub-lethal effect of a substance is not necessarily related to the dose. For instance Kacimi El Hassani et al. (2007)[15] notice that acetamipride, used as a topical application affects the bee locomotion activity at 0,1 and 0,5µg/bee but not at 1 µg/bee.
- The dose having sub-lethal effects for similar substance varies with the considered compartments: Kacimi El Hassani et al. (2007)[15] do not notice any effect from the thiamethoxam in doses < 1ng/bee on the mobility of the bee while a team of the INRA notices some effects of the same substance at 0,5ng/bee on the orientation (Belzunces L., 2008, personal communication)
- The effect can vary according to the age of the bees: Guez et al. (2001)[16] notice that sub-lethal doses of imidaclopride do increase the number of tests needed to remove the extension reflex of the proboscis by presentation of a sucrose solution for young bees (< 7 days), while for elderly bees (> 8 days), the number of essays necessary to create the reflex goes down during the first hour after treatment and goes up four hours later after the treatment.
- The effect can vary finally according to the season: Decourtye et al. (2003)[17] notice less LOEC of imidacloprid (conditioning of the extension reflex of proboscis) to the summer bees than to the winter bees, which seem therefore more resistant to the substance, according to this behaviour at least.

- We also notice that the effect whether contamination affects nectar or pollen can vary because the classes of bees are different. Contamination of the nectar will also quantitatively affect the foraging bees more than the nursing bees. The last ones are more affected by pollen contamination.

The predicted environment concentration PEC

The PEC is based on measurements of the substance and its relevant metabolites in the foraged matrices. This parameter has been established for different types of bees (males, workers, queens and among the workers: nurse bees, foraging bees), which allows taking into account the potential difference of exposure between the different categories of bees.

The predicted non observable effect concentration

The determination of the PNEC brings the necessity of making a series of tests to measure the lowest concentrations that does appear any effect (LOEC). The PNEC are the LOECs assorted by a coefficient of security depending on the accuracy and reliability of the tests. These tests should be made on the concerned categories of bees and on the concerned behaviour (for instance the foraging for the orientation).

Various methods appear in literature, which allow to determine the PNECs. Most of them are methods used in labs. They are concerned with bee brood (bee brood feeding test: Aupinel et al. 2007[18]), bee locomotion, homing flight (see for instance Vandame et al. 1995[19]), the learning abilities assessed through the proboscis extension reflex (Decourtye et al. 2004[20]), the bee thermoregulation, the foraging intensity. The bees lifespan and the queen egg-laying should be assessed too because these capacities are of high biological significance for the hive. The methods' strength is not always attested and some of these should be performed again in different laboratories in order to make or to elaborate ring tests that could be brought into the assessment scheme.

A particular attention should be paid to the effects on the immune capacities since such effects are documented for various substances and microorganisms, some non-pathogenic organisms becoming pathogenic when associated with defined substances.

Immune capacity

With some species of arthropods, the chemical PPPs at very small doses can make the individual sensitive to some pathogens, so that the economic use of such associations is taken into consideration (for instance for termites control). As far as we know, no study has been performed on this subject. However some pathogens (*Beauveria*, *Nosema*) and some substances (imidacloprid) are the same as substances and pathogens possibly present in the hives (Cuthbertson 2005 et al.[21], Feng and Pu 2005[22]). Moreover, some target pests are *Hymenoptera* (termites, leaf-cutting ants - Santos et al. 2007[23]). Pesticides with long-term effects are thus likely to depress the bee's immune capacity.

From another point of view, CCD mortalities seem to go with or to be due to various pathologies, appearing as abnormal in number and in intensity (*Nosema*, virosis….). During eco-toxicology tests performed by PPPs manufacturers, it is observed that treated hives suffer from *Nosema* development or from loss of queens at the end of the test. Researches should be undertaken on bees in this matter (foraging bees and home bees).

Relevance of the field and tunnel tests for systemic PPPs in soil and seed treatment

The assessment of systemic PPPs in tunnels or in the fields have to be made over a long period because these PPPs are likely to contaminate the nectar and/or the pollen. The observations may have to vary from several weeks (observation of the effects on the summer bees) to several years (Cruiser field tests in France). Particularly the evolution of colonies on contaminated stocks during wintertime is a multi-annual fact.

Tunnel tests

Assessing chronic effects requires bee colonies to be confined for a long period of time. Bees cannot bear being prevented from flying, what they usually do over long distances; the colonies development can be affected, such as reported in studies submitted in the authorization reports. For instance, the colonies are completely deprived of brood at the end of the test. This observation does not permit to conclude that the product is harmless since the effect can be masked by the confinement.

The tests in tunnels cannot control the exposure due to the differed consumption. When colonies are put in the tunnel with food frames, it is not assured that the pollen consumption during the test is provided by the contaminated source instead of the combs stocks. Only foraging bees are in contact with the treated pollen that they bring back to the hive, without any effect when the contaminated substances are inside the grains of pollen and not on the surface. Due to these reasons, tunnel tests cannot be considered as a higher level tests in comparison to PEC/PNEC tests when the substance is detected in the foraged matrices, particularly when no toxic standard is available.

Field tests

The tests on the field will raise a double problem: the chemicals background and the difficulty to ensure a representative exposure. In the field the tested substance can interfere with other substances used in the neighbourhood. This requires usually to move the treated hive to a non-agricultural area after the test, but this is artificial compared to the real conditions the field test is supposed to reproduce – this representative reproduction is the justification of the determining character of this test in the assessments. For the same reason, it is difficult to obtain the same conditions between treated fields and controls.

There is no easy way to measure bee exposure, even not through a pollen analysis. For this reason the field tests do not represent a sufficient reliability to be the highest tier tests in the assessment scheme when assessing substances detected in the foraged matrices.

Detection of contaminations through sowing dust

Dust contamination assessment is a general endpoint of PPPs assessment. Current measurements are based on dust gathered in Petri dishes put on a defined distance from the field side under the dominant winds. As a consequence of recent incidents in Germany, Italy and Slovenia, researches are carried out in order to define reliable methods to assess the dust spread. Honeybees and wild bees are likely to be contaminated by contact and through the morning dew harvest. The specific assessment of the risk for bees should take into account this specific exposure.

Synergies

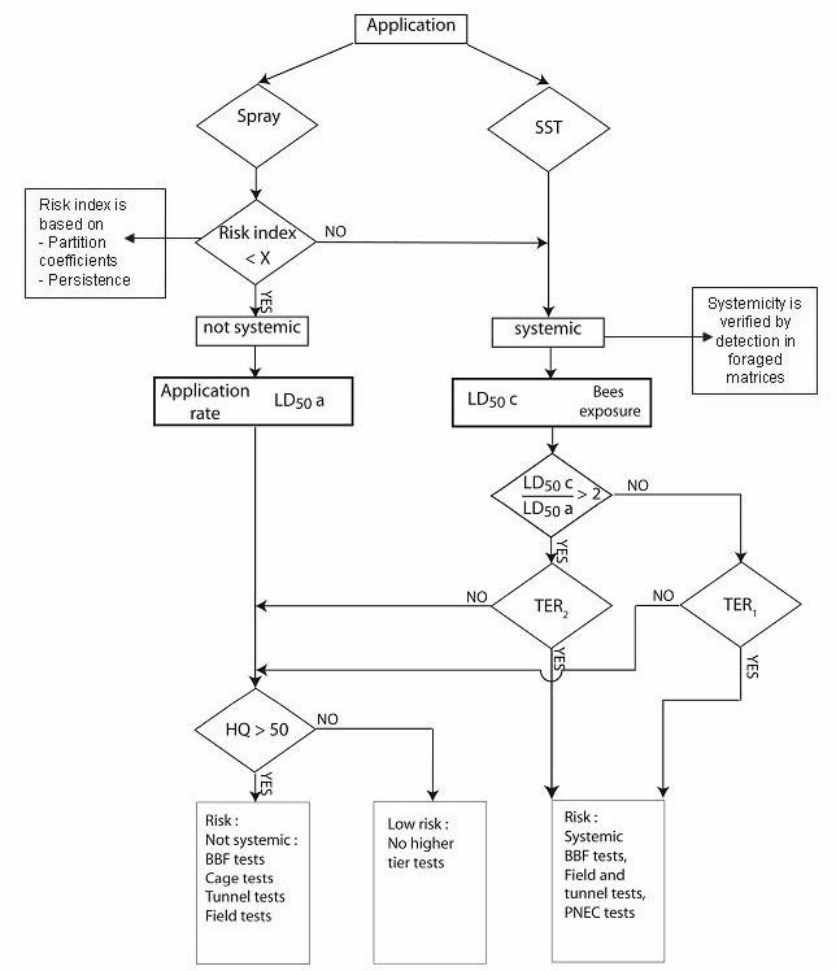
A same substance may have no effects at a certain dose when given alone, but may have a significant impact when associated with another substance. For instance, according to Vandame et al. (1998)[24], deltamethrin doses < 1,5ng/bee do not have effect on the thermoregulation of the bee, while an effect appears when deltamethrin is associated to prochloraz or difenoconazole. The association between insecticides and fungicides is frequently used. The legislation recommends testing the products in realistic conditions as far as possible. The synergies between products regularly associated in cultural practise must thus be tested. Seed coatings made of successive layers of different substances must be considered as a mix as the plant will absorb all these substances together. Synergies are likely to occur.

Reliability of studies

For all the tests we ask that reliability criteria would be established. The CST has established validity criteria for different types of studies (CST, 2004[8], pp. 40: dosage in the pollen and nectar, 51: chronic toxicity, 61: sub-lethal effects, 67: studies in cages,73: field tests).

A guideline should be established for the observation of behaviour in tunnels and fields. It should specify the length of observation time, the identified parameters, the counting method and any other means aimed at gathering objective information.

The quantities of pollen/nectar ingested by the different classes and castes of bees should be defined during an expert debate, on the basis of the 'the worse case' principle.



Conclusions

We propose a risk assessment scheme organised as follows:

- At the first tier, a risk index is calculated based on the persistence and the partition coefficient of the substance (as a indication for systemicity; pKow or pKoa is chosen according to the application mode, e.g. foliar or in soil or seed treatment).

- If the risk index shows a risk of systemicity, the presence of the substance is verified in the foraged matrices.
- If the substance is not present in the foraged matrices, the current assessment scheme is applied.
- If the substance is present in the foraged matrices, the acute mortality and the chronic mortality are measured and a TER is calculated.
- If the TER shows a risk, a complete assessment is performed (including bee brood feeding test and PEC/PNEC assays and calculation).

The process is summarized in the scheme below (see Figure).

Before implementation, further research is needed to determine:

- quantities of pollen consumed by the winter-bees,
- effects of small doses on the bee immune system when the substance is persistent.

The current risk assessment scheme for PPPs is not adapted to systemic substances, particularly those suspected to be present in pollen and nectar. It is important to take into account the persistence and systemicity of the substances, to put these parameters in relation with toxicity and with bee exposure, and to define methods capable of assessing the potential chronic lethal and sub-lethal effects. A fundamental change in the assessment scheme is critical for the survival of bees and other wild pollinators. We hope to have contributed to this reflection.

References

[1] Porrini Cl, Sabatini AG, Girotti S, Fini F, Monaco L, Celli G, Bortolotti L and Ghini S, 2003 : The death of honey bees and environmental pollution by pesticides: the honey bees as biological indicators, Bulletin of Insectology **56** (1): 147-152 (2003)

[2] Claudianos C, Ranson H, Johnson RM, Biswas S, Schuler MA, Berenbaum MR, Feyereisen R, and Oakeshott JG: A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. Insect Molecular Biology **15 (5)**: 615-636 (2006)

[3] Alix A and Vergnet C: Risk assessment to honey bees: a scheme developed in France for non-sprayed systemic compounds, Pest Manag Sci **63**: 1069 – 1080 (2007)

[4] Chauzat MP, Faucon JP, Martel AC, Lachaize J, Cougoule N and Aubert M: A survey of pesticides residues in pollen loads collected by honey bees in France, J. Econ. Entomol. **99** (2): 253 - 262 (2006)

[5] SANCO/10329/2002 : Guidance document on terrestrial ecotoxicology under council directive 91/414/EEC, 17 Octobre 2002, 39 pp. (2002)

[6] Villa S, Vighi M, Finizio A and Bolchi Serini G: Risk assessment for honeybees from pesticide-exposed pollen, Ecotoxicology, **9**: 287-297 (2000)

[7] Halm MP, Rortais A, Arnold G, Taseï JN and Rault S: New risk assessment approach for systemic insecticides: the case of honey bees and imidacloprid (Gaucho), Environ. Sci. Technol. **40**: 2448-2454 (2006)

[8] OEPP/EPPO: EPPO Standards PP1/170(3) Test methods for evaluating the side-effects of plant protection products on honeybees. Bulletin OEPP/EPPO Bulletin 31 , 323–330 (2001)

[9] CST (Comité scientifique et technique) : Imidaclopride utilisé en enrobage de semences (Gaucho) et troubles des abeilles - Rapport final - (undated (probably 2004))

[10] Rortais A, Arnold G, Halm MP. and Touffet-Briens F: Modes of honeybees exposure to systemic insecticides : estimated amounts of contaminated pollen and nectar consumed by different categories of bees, Apidologie **36** :71 – 83 (2005)

[11] Bonmatin JM, Marchand P, Charvet R, Moineau I, Bengsch ER and Colin ME : Quantification of imidacloprid uptake in maize crops, J. Agric. Food Chem., **53**: 5336 – 5341 (2006)

[12] Keller I, Fluri P, and Imdorf A: Le pollen et le développement des colonies chez l'abeille mellifère (1ère partie – 2ème partie), Station de recherche agroscope de Liebefeld-Posieux ALP, centre de recherches apicoles, Liebefeld, CH-3003 Berne, undated, available on the website http://www.apis.admin.ch (accessed 10 June 2008)

[13] Desneux N, Decourtye A and Delpuech JM: The sublethal effects of pesticides on beneficial arthropods, Annu. Rev. Entomol. **52**:81–106 (2007)

[14] Vandame R, Meled M, Colin ME and Belzunces LP: Alteration of the homing-flight in the honey bee Apis mellifera L. exposed to sublethal dose of deltamethrin. Environ. Toxicol. Chem. **14**:855-860 (1995)

[15] Kacimi El Hassani A, Dacher M, Gary V, Lambin M, Gauthier, M and Armangaud, C: Effects of sublethal doses of acetamiprid and thiamethoxam on the behaviour of the honeybee (Apis mellifera), Arch Environ Contamin Toxicol, **54(4):** 653-61(2008)
[16] Guez D, Suchail S, Gauthier M, Maleszka R and Belzunces LP: Contrasting effects of imidacloprid on habituation in 7- and 8- days-old honeybees, Neurobiology of Learning and Memory, **76** (2), 183 – 191 (2001)
[17] Decourtye A, Lacassie E and Pham-Delègue MH: Learning performances of honeybees (Apis mellifera L) are differentially affected by imidacloprid according to the season, Pest Manag Sci **59**: 269–278 (2003)
[18] Aupinel P, Fortini D, Michaud B, Marolleau F, Tasei JN and Odoux JF: Toxicity of dimethoate and fenoxycarb to honey bee brood (Apis mellifera) using a new in vitro standarized feeding method, Pest Manag Sci **63**: 1090 – 1094
[19] Vandame R, Meled M, Colin ME and Belzunces LP: Alteration of the homing-flight in the honey bee Apis mellifera L. exposed to sublethal dose of deltamethrin. Environ. Toxicol. Chem. **14**:855-860 (1995)
[20] Decourtye A, Devillers J, Cluzeau S, Charreton M and Pham-Delègue MH: Effect of imidacloprid and deltamethrin on associative learning in honeybees under semi-flied and laboratory conditions, Ecotoxicology and Environmental Safety, **57**, 410 – 419 (2004)
[21] Cuthbertson AG, Walters KF and Deppe C: Compatibility of the entomopathogenic fungus Lecanicillium muscarium and insecticides for eradication of sweetpotato whitefly, Bemisia tabaci. Mycopathologia. **Aug.160v(1):** 35-41 (2005)
[22] Feng MG and Pu XY: Time–concentration–mortality modeling of the synergistic interaction of Beauveria bassiana and imidacloprid against Nilaparvata lugens, Pest Manag Sci **61**:363–370 (2005)
[23] Santos AV, Lorenz de Oliveira B and Samuels RI: Selection of entomopathogenic fungi for use in combination with sub-lethal doses of imidacloprid: perspectives for the control of the leaf-cutting ant Atta sexdens rubropilosa Forel (Hymenoptera: Formicidae), Mycopathologia **163**:233–240 (2007)
[24] Vandame R and Belzunces LP: Joint actions of deltamethrin and azole fungicides on honey bee thermoregulation, Neuroscience Letters **251** (issue1): 57-60 (1998)

III. Bumblebees and other bee species

The impact of different concentrations of a pyrethroid insecticide on the cyclic gas exchange cycles on bumble bees

*Karise, R., Kuusik, A., Mänd, M., Metspalu, L., Hiiesaar, K., Luik, A.

Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, 1 Kreutzwaldi St., 51014 Tartu, Estonia, *Reet Karise, PhD, reet.karise@emu.ee

Abstract

Minor effects of pesticides may remain unnoticeable in adult bees because of no visible changes in their behaviour throughout several days after coming into contact with pesticides. The hypothesis of this work is that changes which are not observable through the behaviour of the bumble bees can be seen through physiological patterns. The aim of the present research was to study the effect of low concentrations of Fastac 100 EC on discontinuous gas exchange cycles of bumble bee *Bombus terrestris* foragers. Using a system of flow-through CO_2 respirometry, the effect of different concentrations of alpha-cypermethrin on bumble bee foragers was studied. We found that the concentration of Fastac 100 EC that is used in the fields and a tenfold solution of that caused significant decrease in the frequency of bursts of CO_2 releases in bumble bees. 20-fold diluted solution did not cause the significant decrease. The lifespan of treated bumble bees also decreased by the field concentration and ten-fold diluted concentration. Alpha-cypermethrin caused changes in the respiration patterns of *B. terrestris* foragers although not always seen through the behaviour. These changes could potentially lead to a decreasing individual and colony survival.

Keywords: Respiration cycles, pyrethroid insecticide, bumble bees