“Pesticide Risks to Wild Pollinators”

Workshop report

30th October – 1st November 2011
Wageningen University & Research Centre
The Netherlands

Project: “Knowledge management of pesticide risks to wild pollinators for sustainable production of high-value crops in Brazil and Kenya” (BO-10-011-113)
Table of Contents
1. **Objectives**

The third workshop on Pesticide Risks to Wild Pollinators took place from 30th of October till 1st November, 2011, at Wageningen University and Research Centre. The workshop was called to consolidate the research findings of a project funded by the Dutch Ministry of Agriculture, Nature and Food Quality on “Knowledge management of pesticide risks to wild pollinators for sustainable production of high-value crops in Brazil and Kenya” (BO-10-011-113)

The project was initiated by Wageningen University and Research Centre at the request of the FAO Global Action on Pollination Services for Sustainable Agriculture, within the framework of the International Initiative for the Conservation and Sustainable Use of Pollinators (IPI) established under the Convention on Biological Diversity. Partner countries in the project are Brazil and Kenya (see section two for detailed information).

The main objectives of the workshop were:

i. to share results of the acute toxicity tests (LD₅₀);
ii. to discuss the assessments of pesticide risks to wild bees in the focal crops;
iii. to discuss the various risk assessment models that could be applied to wild bees; and
iv. to determine progress of the project and possible knowledge gaps.

The programme of the workshop is provided in Annex 1.

2. **Participants**

The workshop was attended by the following participants:

**Brazil**: Roberta Nocelli (Universidade Federal de São Carlos), Márcia Ribeiro (Embrapa Semiárido), Breno Freitas (Universidade Federal do Ceará), Stephan Carvalho (Universidade Estadual Paulista)

**Kenya**: Gladys Maina (Pest Control Products Board), Chris Odhiambo (National Museums of Kenya), Muo Kasina (Kenya Agricultural Research Institute), Paul Ngaruruiya (Pest Control Products Board),

**FAO**: Barbara Gemmill-Herren (Plant Production and Protection Division)

**Netherlands**: Tjeerd Blacquière (Plant Research International/WUR), Irene Koomen (Centre for development Innovation/WUR), Ivo Roessink (Alterra/WUR), Harold van der Valk (Pesticide management consultant), Jacoba Wassenberg (Ctgb/Dutch Pesticide Registration Board)

Contact details of all participants are provided in Annex 2.
3. Update on meetings attended by partners in 2011

ICPBR Cholula meeting – Barbara chaired a session on pesticides and non-Apis bees. Several posters of the team were presented there. Conference proceedings, for which David Roubik is general editor, will be published in 2012, including ideas around a structured risk assessment, linking bee ecology and pesticide effects. So far, sub-lethal and combi-tox have not been considered yet. Note: ICPB(ee)R will be called ICP(ollinator)R in future.

SETAC Pelliston Workshop, Pensacola: Pesticide risk assessment for pollinators – was attended by Roberta and Muo. The association brings together the scientists, regulators and the pesticide industry to develop and agree on matters related to pesticide assessments. The objective of this meeting was to develop new guidelines on pesticide risk assessment for bees. For the first time non-Apis bees were considered. The meeting was organised in three panels; Lab tests, semi field and field tests, and, the risk assessment model group. A fourth group, Non Apis bees comprised of experts working on non Apis bees but these were distributed amongst the three groups to provide expertise for non Apis consideration. Quite interesting in the meeting was the lack of open communication of outcomes across the groups. Participants agreed to keep to each group and communication of the meeting outcome was to come from the SETAC secretariat. A book will be published early 2012 with contents of the groups. A summary of the outcome has been published (see: http://www.setac.org/sites/default/files/executivesummarypollinators_11oct2011.pdf#overlay-context=node/265).

Barbara noted that there was quite a lot of discussion about the Apis tests that are being used. She also noted that there are doubts about the extrapolation of tests between crops, and also that honeybees forage outside the test field putting doubts on some of the test results.

Note: some wild bees forage over a smaller range and might therefore be more suitable for field testing.

SETAC meeting, Milan: Ecosystem Protection in a Sustainable World: a Challenge for Science and Regulation – Ivo presented the first results of the toxicity tests for dimethoate.

The Second International Steering Committee (ISC) Meeting of the Global Pollination Project, “Conservation and Management of Pollinators for Sustainable Agriculture, through an Ecosystem Approach”, Ghana – Chris presented the first year results of our project. The 3rd ISC meeting will take place in Nepal this November. Both Barbara and Chris will attend and present a summary of the progress in toxicity tests and risk profiles.

OECD-PEIP – Jacoba is one of the Dutch contact persons. Wild bees are included, inventory of test is made to establish missing tests.

Pesticide registration meeting, Canada – Paul reported that globally a profusion of tests is being used. One of the recommendations from the meeting was that FAO and OECD should work together. IPM should be used all over the world.

Apimondia, Buenos Aires – Tjerd and Márcia attended. A small part of the meeting was dedicated to non-Apis bees. Compared to the last meeting in Montpellier there was little attention to neonicotinoids.
Forthcoming meetings: in 2012 there will be an Apimondia symposium about bee flora in San Marino. Koos Biesmeijer & Barbara will be doing some of the chairing. A session about pesticides would be one of the possibilities.

Other information that was shared

Introduction new participants from Brazil:
Breno introduced himself as a bee expert, not a pesticide expert. He organises an international, two-week ecology research course which was held in Amazonia this year. Attention is given to the effect of pesticides on bees. Having involvement of African partners would be a valuable addition. As part of the pollinator initiative he has written a review (in Portuguese) about effect of pesticides on pollinators. Focus is mainly on Apis but includes a review of other species. Attention is given to good practices for farmers and beekeepers. (FAO will see if a translation of the document can be made). In addition, he has published a guide book (in Portuguese) about rearing of carpenter bees. He distributed some copies to partners in the meeting.

Stephan: works on biomarkers to determine the effects of thiamethoxam, fipronil, spinosad, deltamethrin on Apis spp. Focus is on enzymes in detoxification systems and oxidative stress. The time of exposure is important but it is difficult to extrapolate to the field situation.

From Brazil there is currently nobody who presents the competent authority. The Brazilians explained that there is not clearly one competent authority, responsibilities lies with 3 ministries (Ministry of Agriculture, Ministry of Environment and Ministry of Health).

Awareness creation in Kenya:
The team in Kenya has organised several trainings, partly for the extension services but also meetings with farmers’ groups. There is a lot of interest, importance of IPM is emphasised but also the phasing out of the most toxic pesticides needs attention.

EFSA:
A panel, chaired by Robbert Luttik of the RIVM, Netherlands, will review bee toxicity tests on request of the commission.

Other:
The University of Cape Town offers a postgraduate diploma course in pesticide risk management. One of the required reading articles is one that Muo wrote for the KARI Biennial Scientific Conference in 2010.

Tjeerd has recently written a review on impact of neo-nicotinoids on bees.

4. Acute toxicity tests & Species Sensitivity Distribution

Contact toxicity tests by droplet (application on thorax) were conducted with deltamethrin. The bee species tested were:

Brazil: Africanized Apis mellifera and Scaptotrigona postica (stingless bee)
Kenya: Apis mellifera scutellata, Meliponula ferrunginea
Netherlands: *Apis mellifera mellifera* (European honeybee), *Bombus terrestris* (bumble bee), *Osmia rufa* (mason bee)

In Brazil the tests with *Melipona scutellaris* will be conducted as soon as the weather allows this. It had been a very cold, wet winter and an unpredictable spring. The *Osmia rufa* testing in the Netherlands gave a value that was clearly outside the expected range (LD$_{50}$ of 312.607 compared to that of the other species that did not exceed 2.2). This test will be repeated in 2012.

For dimethoate there appears to be a good correlation between size and toxicity (microgram per insect), the smaller bees appear to be more sensitive. This does not hold for the results of the deltamethrin tests. The same appears for level of social behaviour, the more social the more sensitive, there is a correlation for dimethoate but not for deltamethrin. Considering the challenges faced in 2011 tests in the three countries, it would be wise to repeat the experiment. In Kenya, there was prolonged drought and bees were likely to be stressed due to lack of food. Also, there were periods of extreme weather conditions affecting bees in the said period.

*Apis mellifera mellifera* is, in both tests, one of the most sensitive species. On this basis, limited data appears that for the contact toxicity testing; it might be tentatively concluded that *Apis mellifera mellifera* is a good indicator. However, more tests might be required to confirm this considering the inconsistencies of the second ring test.

**Discussion:**
It was agreed that this test is a good start to indicate pesticide hazard to bees. The relevance of lab tests to field exposure is was queried, for example; do laboratory tests cover the exposure in the field (e.g. space, behaviour, technique of application)? How do tests on individual bees relate to effects of exposure of groups, for social bees there could well be a big difference. Felipe Contrera studied group effects and mortality of stingless bees. Duration of test and if tropholaxis occurred was discussed. It was concluded that the duration 3 times 24h was such that no tropholaxis occurred.

About the method, maybe also contact toxicity test by walking on exposed surface is necessary. Exposure will be difficult to determine but would be related to exposure to residues on plant surfaces.

What is needed to confirm the tentative conclusion of *Apis mellifera mellifera* to be a good indicator? Since there are hardly any results for solitary bees the first thing to do would be to test more bees. All teams have still a few more tests to be conducted. Once these results are available further conclusions can be drawn. Ivo will coordinate this.

5. **Assessment of pesticide risks to wild bees in the focal crops**

On the basis of a survey format all three countries represented in the project had been asked to provide data on the selected focal crops in their respective countries. For Brazil this was tomato and melon, for Kenya, tomato, French beans, coffee and cucurbits, and for the Netherlands tomato (under glass) and apple. The aim of this was to understand how the pollinators are exposed, i.e. the basis of a risk assessment model.
Data already available are summarised in the working document, see Annex 3. Harold explained that the information available is not sufficient to develop a risk assessment model but can be used to develop a risk profile i.e. which exposure factors are likely to influence risk in cropping systems.

The three countries have completed the survey in a different way. The approach taken in each country was explained.

**Brazil**

For melon: meeting with all people involved in the pollination project. Information was sourced from the literature (not an extensive search) and practical experience, and also from crop producers.

For tomato: a small literature review was conducted and meeting with some professors were held. Database on pesticides was consulted and input from tomato specialist was also taken into account.

Problems which occurred with filling in the survey: 1) Crop producers were reluctant to provide information on the pesticides they are using (esp. on mixtures and dose rates). 2) Lack of information on how long bees spent outside the nest for foraging, esp. solitary bees. Not all possible pollinators may be present in the crop because of intensive spraying regime.

In some cases spraying is carried out every other day. Many pesticides are applied, sometimes 17 products in a tank mixture, as a ‘preventive’ measure. Farmers do not regard the risk to pollinators in their spraying regime.

Farmers, in the region of melon crops, receive technical assistance. But many times they do not follow the advices, and/or, receive biased advices (some technicians are also employees of pesticides’ shops and earn more in case they sell larger amounts of products).

In Brazil, sometimes pheromones are used for e.g. whitefly control. These are also attractive to bees and are used to counteract the negative effects of pesticides, attracting the bees back into the field.

Data on amount of pesticides consumed per year and country, showed than Brazil became the major market of pesticide use, billing USD 7.30 billion/year. The second is the USA with USD 6.00 billion/year. However, it is important to explain that Brazil with a tropical climate can have multiple harvest per field in a year. If pesticide use (US$) is converted to amount used per quantity of food, Brazil take the 6th place, preceded by USA, Argentine, EU (except France), France and Japan. On the other hand, pesticide use in Brazil increases much more than the production itself, with production area staying the same.

**Kenya:**

In addition to information gathered from the literature, expert judgement, and information from the pesticide registration office, a questionnaire for farmers was developed – Farmer knowledge on flower visitors and types of pesticide use. This questionnaire was taken to three study Counties: Kirinyaga and Machakos (horticultural: French beans, tomatoes, cucurbits; all small scale farmers) and Kiambu (coffee).

Farmers named many insects as flower visitors, not only pollinators but also pests and predators. Negative effects on bees (direct acute mortality, but also decline over the years) are sometimes observed. Increased level of flower abortion may be observed but farmers do not connect this with decreased pollination. Farmers sometimes avoid spraying bees to
protect honey provision but not to protect pollinators (for residue reasons?). A case was also noted in coffee where farmers avoid to spray during flowering to avoid flower abortion but not necessarily to protect bees.

Agrochemical stores, responsible for sales of pesticides, are the main source of information for farmers on how to apply pesticides. Additional information is obtained from other farmers and agricultural extension service providers. Most farmers apply insecticides from the basis of pest infestation. However, for diseases it is mainly calendar based as they understand the stage of development when specific disease sets in, thus sprays are preventive measures. In regard to the knowledge of bees; honey bees are associated with honey, carpenter bees are seen as a pest of roof timber and sometimes even as a cause of flower abortion, there is no associations with pollination. At times carpenter bees are actively sprayed to control them.

Farmers use product labels so this may be a good place to provide guidance on pollination risk mitigation. Information on hazard to bees is already on the label. More education is needed on interpretation of risk mitigation (when exactly are bees foraging etc.). This additional guidance, next to general information, may need to be focussed on specific crops and may even area specific. There was no problem in obtaining information on pesticides use by farmers (compared with Brazil).

Note: Further analyses will be carried out on the Kenyan farmer surveys and results published in a peer reviewed journal.

The Netherlands:

Information was collected by meeting with a small group of experts for both crops. Some gaps were then filled with information from the literature. Pesticide use data were collected from Dutch statistics office (driven by anonymous questionnaire, filled in truthfully, also illegal pesticides popped up). This gives an overview of overall data for the Netherlands, not specifically per farmer. Authorised pesticides per crop will be made available by the nVWA.

Problems with filling in survey: Biology of managed bees is well known but for wild bees this is not.

From discussion of the risk profile document which combines inputs from the three countries, the following issues arose:

- Definition of systemic with relevance for bee risk is needed (e.g. discussion arose about dimethoate if it was systemic or not. Note: a quick search establishes that dimethoate is actually a systemic as well as a contact insecticide;
- The size of the field and/or the percentage within an area in which a crop is grown may be important – if larger, pollinators are likely to focus more on that particular field for foraging. May differ within crop (i.e. type of cropping system);
- Smallholder system: probably other crops or margins available so alternative place for foraging and refuges will be available for bees;
- Despite the intensive spraying regime there are still pollinators present in large cropping systems in Kenya; and not only honeybees, but also wild bees are present;
- In Brazil, ‘large’ cropping systems, which are much larger than those in Kenya, even there non-Apis are still present (there is fast recovery from off-field areas after spraying);
- Consumer are demanding better agricultural practice, mainly because of concerns for residues;
Flowering time: some crops have mass flowering – then many bees may depend on that particular crop completely; some crops always have some flowers present and then the exposure risk may be lower because bees will probably have other foraging sources as well (but risk mitigation might be more difficult);

Some pests require treatment during flowering for example against flower thrips. A management option could be to apply the insecticide just before flowering;

Managing the application time is sometimes logistically challenged. Contract sprayers will appear when it suits their schedule irrespective of best time of application to protect non-target organisms;

The risk mitigation that we propose has to be manageable in practice. E.g. spraying during the hottest time of the day may not be good in Kenya, in Brazil as well, depending on the region;

The weather and other environmental circumstances may influence both the behaviour of the bees and the (dissipation of the) pesticide;

In Kenya it is already recommended for all pesticides which are harmful to bees that spraying should not take place when bees are present. But the interpretation of this for farmers is still difficult so this needs more guidance. But the theoretical exercise that we do now (risk profile) is still useful because we may identify crop-specific solutions;

Storage of nectar and pollen in nest: if stored longer maybe the risk is lower because of breakdown of substances but this could also be an increased risk because the colony is exposed for longer and also can be more spread, since larvae (of several generations) can be also contaminated;

Some adult bees feed on pollen as well as on nectar, but most do not (the amount consumed is usually smaller);

Ratio female/offspring: not parameter for exposure, but important for possibility for recovery (of the colony, for social bees);

Amount of pollen taken in is different for males and females;

No population dynamics yet included, list is only focussed on individual bees;

Which parameters are important in the life history paragraph has not been decided yet.

The conclusion on high and low risk in Table 4 – crop factors - was discussed. Especially for coffee it was deemed that the conclusion that there was not a high risk of exposure of wild pollinators in the crops was not sufficiently founded. It was thought there to be a risk for all of these crops. It is not necessary to rank the crops, but the parameters may be ranked for exposure within the crops. All the factors will have to be considered when assessing the risk in the specific crops. Weighing factors to determine relative importance of different parameters will need validation and input from many experts, so might be very difficult to determine.

Conclusion: the overall exposure line in the table will be taken out. And there could be a statement added per factor on its relative importance to exposure and an explanation on the reasoning why.

Tables on bee biology: there are many knowledge gaps, but it is possible to qualitatively compare the risk to the different bee species.

Several uses for this survey were identified:

- If toxicity data are available, the risk profile document can be used to compare the risk of different products. From here the low risk products can be given preference for use and, in the EU, will comply to requirements for the sustainable use directive.
- Prioritise crops in which you need to do further work first (research and risk mitigation) at the national level. For the prioritisation the importance of pollination for that particular crop should be considered as well.
- Give information on a particular crop - highlight which issues are still unknown.
- The project is focussed on pollination – food production. Additional output may be that there is more attention for biodiversity, but that is not our main goal at present.
- Risk mitigation – prioritise within crop.
- Highlight which areas of agricultural practice can be improved with respect to bee risk but that is mainly information gathered through the survey that Kenya used, not information readily available in the general document.

Breno felt that industry should be asked to provide much more information on the risk to pollinators and to educate pesticide users. There should be a much greater responsibility on this with the industry. It was however felt that this is a joint responsibility because the (data) requirements are set by the governments and the authorisations, including risk mitigation, are also set by governments.

*How to proceed with the risk profile document:*

Harold will finalise the draft report on risk profile, send it round for comments and gap filling and finish it before the end of the year. The aim is to produce a report which will be made available through FAO so that other countries can also set-up a similar survey.

*Database*

There is a database on acute contact and oral toxicity for honeybees which is available online by Oregon University. Barbara will send the link to one and all. For bumble bees there’s a review by Veerle Mommaerts and Guy Smagghe of the University of Ghent. It was discussed if it would be possible to compile a database on the toxicity data on wild bees (non-Apis and non-bumblebees)? The conclusion was that this would be very useful, and could also be published.

At the ICPBR symposium it will be checked whether any other organisation (SETAC, OECD etc.) is already setting up a similar database. If not, we will gather information (publications and/or information from researchers) as a first step. There is for instance a review available from Brazil, Roberta will send the article (in English).

To share publications, an internet, wild bee library through Mendeley (‘poor people’s endnote’) can be set-up. As long as this is a closed system, there are probably no implications regarding copyright but this should be checked. Setting up a proper database will however require further funding. Harold will coordinate these activities.

6. **Comparison of risk assessment methods**

To be able to decide on which approach to take for pesticide risk assessment of wild bees it was felt necessary to have a look at available models. A document (see Annex 4) was compiled by Harold in which the various methods are described. This document was discussed.

Stephan knows of a computer model for risk assessment which is being developed in France. Contact is Axel Decourtye, it is possible to obtain authorization through ACTA.

*Way forward for risk assessment:*

OECD and EFSA need to be contacted to ensure that we do not have overlapping activities and to determine if either organisation will come up with a risk assessment method for wild
bees. Whichever method will be decided on, there will always be a need for more semi-field / field studies to validate the model.

Note: Barbara may know a donor who wants to fund a PhD in Kenya to study stingless bees and pesticides. Looking at field trials might be incorporate in the projects.

Another idea that was discussed was that it might be opportune to write a review for risk assessment. This will however be a major task and is not part of the current project.

7. Deliverables work plan 2011

- Comparative toxicity data generated on different bee species resulting in a species sensitivity distribution (SSD), which will contribute to the generic pesticide risk assessment model [target group: pesticide regulators]

See point 4, this report. Roberta, Muo, Ivo & Sjef will finish the tests as far as part of this work plan. The Kenyan team is keen to carry out some observations on flying in and out of the nest as discussed in Nairobi November 2010.

- Joint peer reviewed scientific paper on the SSD. (Target group: scientific community, policy makers)

Ivo and Sjef will coordinate the writing process for the scientific paper on the SSD. Timing of this is still to be considered. The initial results are already floating around in various other fora. It might be opportune to write an initial paper as soon as possible, followed by a paper on the full set of results.

- First version of a generic pesticide risk assessment model for wild bees [target group: pesticide regulators]

Instead of the risk assessment model the risk profile model will be published because there is simply not enough information for a risk assessment model at this stage. This will also mean that the target group will change, the new target group are a variety of stakeholders in other countries that are interested to perform a similar exercise. See also point 5 & 6, this report.

- To anticipate the availability of pollinator sensitivity data, draft mitigation measures will be proposed for the pilot crops (e.g. tomato), based upon expert consultation

Guidance on risk mitigation measures that can be applied to reduce the risk of pesticides on bee pollinators (target group: bee keepers, farmers, other pesticide users);

Mitigation measures for focal crops per country will be produced as leaflets. Irene and Harold will make a first draft and send it round for comments. The draft leaflets should be checked with local beekeepers' and crop producers to see if the recommended measures are realistic, feasible, understandable, and do not contradict local bee behaviour. The pesticide industry is another stakeholder to be consulted, guidance can be provided to them on their role in risk mitigation (part of the industry’s’ stewardship). Another target group is the pesticide sellers since they will have to communicate the guidance. Government institutions should keep the lead in deciding the content of the leaflet. A detailed and more extensive document should be written so this can be used by extension staff etc.

1 It was deemed that one of the challenges would be to reach the group of beekeepers but also a more general misconception of the role of bees in crop production.
In Kenya there are two possible places to publicize the information, in Environment and in the Mazingira magazine. In Kenya there are strong grower associations which have a strong lobby. If these growers’ association start advocating importance of pollination, this will help a lot.
In the Netherlands LTO, the combined Dutch producers organisation, published a short leaflet this year focussing on honeybees and bumblebees and highlighting again that you should read the label. This is in response to a renewed discussion of the effect of neonicotinoids on bees.
Deadline: draft versions, at least for one crop for each country, to be ready by the end of the year.

- Policy brief on the reduction of pesticide risk to pollination services and biodiversity (target group: policy makers)

Even though there are not many data it was decided that an awareness creation to policymakers should be carried out. The challenge with this is to formulate the brief in such a way that the importance of the risk of pesticides to pollinators is communicated clearly. FAO has done a policy analysis in various countries to identify relevant departments and/or people. We can use this analysis for targeting our message. It is important to realize whom we are addressing, various ministries like environment and agriculture are involved.

In Brazil there are good contacts with the environmental ministry, very open to contact with regards to our project and there is a strong interest in more information. An option would be to hold a workshop with the ministry to decide what their role could be.

Action policy brief: Tjeerd with support of rest of the Dutch team will make a first draft and send it round for comments. There will be a general section and a national specific section. Draft ready by the end of this year.

- Proposal for further collaboration

The changing funding environment in the Netherlands is explained. In future this will be linked to the private sector. BOCI projects, funded through the embassies, will not continue. Only funding from the government will be given to research institutes if also the private sector is willing to invest.

It was discussed if there would be possibility of extended funding for wrapping up this project, until half 2012. This is important so that the reporting, which is the most important part, is not rushed.

It was decided to contact the permanent representation in Rome (Mrs. Gerda Verburg, Barbara, Harold & Irene), and the agricultural councillor in Kenya (Kenyan team) and Brazil (Brazilian team). A good summary of progress is needed before the representatives are contacted.

For other sources of funding the following potential donors were mentioned:
- Rabobank are already active and have written a report on pollination. Action: Tjeerd and Harold to contact the Rabobank.
- EU: STD3 - Gladys will look into the possibilities.
- FP8 – for future project but now it might be an opportune time to suggest this as a topic for FP8. Action: Barbara.
- In Kenya there is strong private-public partnership. FPEAK (private sector) has partnered with KARI to develop Practical Training Centre for farmers through a grant from The Netherlands. Other donors have shown interest in additional funding. In addition, FPEAK,
KARI and Jomo Kenyatta University of Agriculture and Technology (JKUAT) have competed for funding through a Niche programme. These projects provide opportunities to implement some of our project recommendations such as awareness creation. In addition, such partnerships can be formed locally to seek funding by The Netherlands foreign mission since their approach has now focused on private funding. Action: Muo.

- KenyaGAP provides an opportunity to take our data to practice and educate farmers. Action: Gladys.
- FAO might in the future have an opportunity to incorporate wild bees into the ‘Save and grow policy’. Soon a new DG of FAO will be in office. He is a Brazilian national (Graciano) and has a background in environmental sciences. Action: Barbara.

Note: For any funding meetings we need the policy brief.

A draft proposal/ programme document will be compiled by Barbara (in 2012). This document will describe the overall umbrella, the big picture which will be helpful in funding discussions. The idea is to write this in a modular format so potential donors can buy into one of the topics.

General components of GEF (global environment facility) funded Global Pollination Project can be used for this proposal:
1) Extension of knowledge base
2) Adaptive management e.g. risk mitigation guidance
3) Capacity building
4) Public awareness and policy mainstreaming

ad. 1) Knowledge base: generic models; obtain info on bee biology & ecology; semi-field and possibly field trials with non-Apis bees both social and solitary; continuation of laboratory work; explain trade-off between pollination and pesticide use (crop vs. environment, food safety including also residues to honey)
ad. 2) Literature review of risk assessment models for bees; develop tool for risk assessment for regulators; develop tool for extension services/farmers; risk profile document; practical advice on risk mitigation for e.g. farmers
ad. 3) Creating awareness with farmers, educating them (make sure that information is understood in the whole chain of people involved); work together with regulators to develop tools for risk assessment and mitigation; improve general knowledge on pollination (in schools, e.g. by young farmers club);
ad. 4) Engagement of policy makers (very carefully); engagement of other stakeholders; create enabling environment; link to good agricultural practice.
8. ICPBR symposium

All participants attended the ICPBR symposium Hazards of Pesticides to Bees. The output from the project activities were presented in one of the sessions (see below). The papers will be published in symposium proceedings as a special edition of the Julius-Kühn-Archiv in Germany.

5. Session - Bumblebees and other pollinators. Chair: Irene Koomen.
7 presentations, 4 posters
11:30 – 13:00
- Introduction by Irene Koomen
- Stephan Carvalho, Thaisa Rout, Andrigo Pereira, Elaine Silva-Zacarin, Roberta Nocelli and Osmar Malaspina: Brazilian bee loss
- Muo Kasina: Bees require protection for sustainable horticultural production in Kenya

13:00 – 14:00 Lunch break. Then continuation session 5
14:00 – 16:00
- Andrigo M. Pereira, Roberta C. F. Nocelli, Osmar Malaspina, Odair C. Bueno: Side-effect of acetamiprid in adult Africanized honeybee
- Tavares Lourenço, Clara; Malfitano Carvalho, Stephan; Malaspina, Osmar; Nocelli, Roberta Cornélio Ferreira: Determination of LD_{50} of fipronil for bees Melipona scutellaris
- Jozef van der Steen, Ivo Roessink, Muo Kasina, Mary Gikungu and Roberta Nocelli: Is the European honeybee (Apis mellifera mellifera) a good representative for other pollinator species?
- Harold van der Valk, Irene Koomen, Tjeerd Blacquière, Marcia de F. Ribeiro, Roberta C.F. Nocelli, Muo Kasina, Mary Gikungu, Jacoba Wassenberg, Sjef van der Steen: Aspects of determining pesticide risks to wild bees – implications for risk mitigation and risk assessment

Related posters:
- Siqueira, Kátia M.M.; Kiill, Lucia H.P.; Coelho, Márcia S.; Araújo, Diêgo C. S.; Gama, Diego R.S.; Lima Jr, Ivan O.; Ribeiro, Márcia F.: Effect of agrochemicals in the pattern of visitation of Apis mellifera in Cucumis melo
- Veerle Mommaerts, Linde Besard, Gamal Abdou-Alla, Guy Smagghe: Assessment of lethal and sublethal effects by spinetoram on Bombus terrestris
- Ivan Meeus, Dirk de Graaf, Kris Jans, Guy Smagghe: Multiplex PCR detection of slowly-evolving trypanosomatids and neogregarines in bumblebees using broad-range primers
- Ivan Meeus, Dirk de Graaf, Guy Smagghe: Detection of viral replication in bees
Annex 1 – Workshop programme

Sunday, 30th October

13:00 – 17:00 Sinderhoeve, Renkum
(participants will be collected from Hof van Wageningen at 12:30)
  – Shared lunch
  – Opening, introduction participants;
  – Update of meetings attended and presentations held;
    o Cholula meeting, short presentation about chapters that result from this
      (Barbara, Roberta, Mary)
    o Other meetings attended?
  – Results of LD50 tests;
    (Ivo, Sjef, Muo, Roberta)

Monday 31st October
9:00 – approx. 17:00
  – Assessments of pesticides risks to wild bees in the focal crops;
    (Harold)
    o Explanation by each team on how data for risk factor assessment were
      compiled and tentative conclusion;
    o Presentation and discussion on risk factor assessment comparison of the
      focal crops;
  – Risk assessment model (see Annex 1);
    (how far are we, what options are there, how does the model relate to activities of other
    groups) (Harold)

Lunch 12:30 – 13:30 Restaurant of the Future
  – Determining knowledge gaps;

Tuesday, 1st November
9:00 – 12:30 (WISL 1.004)
  – Other issues (below outputs as taken up in work plan 2011)
    o Comparative toxicity data generated on different bee species resulting in a
      species sensitivity distribution (SSD), which will contribute to the generic pesticide
      risk assessment model [target group: pesticide regulators];
    o Joint peer reviewed scientific paper on the SSD. (Target group: scientific
      community, policy makers)
    o First version of a generic pesticide risk assessment model for wild bees [target
      group: pesticide regulators];
    o To anticipate the availability of pollinator sensitivity data, draft mitigation
      measures will be proposed for the pilot crops (e.g. tomato), based upon expert
      consultation
o Guidance on risk mitigation measures that can be applied to reduce the risk of pesticides on bee pollinators (target group: bee keepers, farmers, other pesticide users);
o Policy brief on the reduction of pesticide risk to pollination services and biodiversity (target group: policy makers)
  - Proposal for further collaboration (also from the work plan):
  o A full project proposal will be developed to set up a system of pesticide risk management for wild pollinators, which can be used to build upon for wider use in other countries:
    - ICPBR symposium presentations, practice session;
  - Final discussion and way forward.

Lunch 12:30 – 13:00

Depart 13:00 Visit to Syngenta in Weert
(see: http://www.syngenta.com/global/bioline/en/Pages/home.aspx)

Wednesday 2nd November – Friday 4th November
  - ICPBR symposium Hazards of pesticides to bees, Wageningen, the Netherlands
## Annex 2 – Participants

### Brazil

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Annex 3 – Draft Risk Profile Document <which version shall we include?>
Annex 4 - Risk assessment – bees

Below is a (partial) review of risk assessment approaches and methods for honey bees and wild bees. The review is not exhaustive, and if more methods are known to you, please add them to the list. The risk assessment methods have been subdivided in:

A. Regulatory approaches: methods presently used for the registration of pesticides (so far described for EU, USA and Australia – all for honey bees)

B. Other published approaches: risk assessment methods published in the scientific literature or by extension services, etc. These are mainly for honey bees, but a few risk assessment proposals for wild bees are also listed.

C. Other possible approaches: methods not (yet) applied for bees, but used for other non-target arthropods, or in other risk assessment frameworks, and which might be adapted to wild bees.

Please note that, so far, the review has been written rather in "telegram style"; hopefully this is understandable.

Most of the referenced articles and reports have been uploaded on the project's share website, for easy access. [https://portal2.wur.nl/sites/PollinatorRisks/default.aspx – folder Pesticide Risk Assessment]

A. Regulatory approaches

1. EU/EPPO

   Principle
   Tiered approach to assessment of risk from sprayed treatments and from soil & seed treatments to honeybees.

   Assessments: Sprayed treatments – 1st tier

   Calculation of Hazard Quotient:
   HQ = application rate (g/ a.i./ha) / (acute or oral) LD_{50} (μg a.i./bee) (= empirical risk assessment procedure)

   Input data required:
   Application rate; LD_{50}

   Trigger value:
   HQ < 50  →  low risk

   Validation of trigger value:
   Empirical, i.e. based on reports of honeybee poisoning incidents and associated field application rates in the UK (Aldridge and Hart, 1993; Mineau et al., 2008) and in the UK, Netherlands and Germany (Defra, 2009).

   Possibility for use for pesticide risk assessment of non-Apis mellifera bees:
   Toxicity data
• LD₅₀s of non-Apis mellifera bees; or

• Extrapolation factor for difference in LD₅₀ values between Apis mellifera and other bees based on toxicity test data. (e.g. see Thompson and Hunt (1999), and van der Steen et al. (2009), for bumblebees)

Exposure data
• Application rate can be maintained

Trigger value for HQ
• Empirically determined: i.e. this incorporates data on exposure of honeybees as well as impact of the pesticide at colony level (= behaviour, pesticide fate, population effects/dynamics, environmental conditions, etc.)

• To empirically set a specific trigger value for non-Apis mellifera bees would require a large set of field data on mortality. This is not likely to be possible.

• Applying an assessment factor to “correct” the honeybee HQ trigger:
  • might be done on the basis of theoretical exposure/effect modelling of different groups of bees and compare with a similar honeybee model.
  • might be done on the basis of “best expert guestimate” (e.g. “time-out-of-nest” – Roessink & van der Steen in prep?)

Assessments: soil & seed treatments – 1st tier

Calculation of Toxicity Exposure Ratio:
TER = LD₅₀ (µg a.i./bee) / ETE₀₉₀

ETE₀₉₀ = Residue₀ * MFI

with:
ETE₀₉₀ = estimated theoretical exposure of bees to pesticides in pollen or nectar (µg a.i./bee)
Residue₀ = Residues in pollen or nectar of the relevant crop (mg a.i./kg) (default worst case = 1)
MFI = the maximum daily food intake by a honey bee (g/bee) (default maximum = 0.128)

Input data required:
LD₅₀ (residue levels in pollen); (daily food intake by bee)

Trigger value:
TER ≥ 10 ➔ low risk

Validation of defaults and trigger value:
Residues in pollen or nectar: based on residue measurement in all types of plant parts, including very limited pollen en nectar data: 95% percentile = 0.55 mg/kg (Alix & Lewis, 2010; referring to Alix et al. 2009, which appears to be based on Defra 2009)
[Note: more pollen residue data appear to be available now, following various neonicotinoid reviews]
**Maximum food intake:** based on Rortais et al. (2005)

**Extrapolation factor of 10, for acute to chronic effects:** based on acute-to-chronic ratios for a limited number of substances (Defra, 2009); but needs further work to confirm correlation (EPPO 2010).

**Possibility for use for pesticide risk assessment of non-Apis mellifera bees:**

**Toxicity data**
- LD$_{50}$ of non-Apis mellifera bees; or
- Extrapolation factor for difference in LD$_{50}$ values between *Apis mellifera* and other bees based on toxicity test data. (e.g. see Thompson and Hunt (1999), and van der Steen *et al.* (2009), for bumblebees)

**Exposure data**
- Residue level in pollen/nectar can be maintained as for honeybee
- Maximum food intake rates would need confirmation for other species.

**Trigger value for HQ**
- Based on limited honeybee data (extrapolation from acute oral LD$_{50}$ to 10-day chronic LD$_{50}$)
- May be applicable also for other bee groups, but should be confirmed at some stage.

**References**

*Description of the risk assessment method*


**Others**


2. US-EPA

Principle

Tiered hazard assessment based on acute contact toxicity. Currently, EPA does not characterize residue exposure for honey bees and other beneficial insects.

Assessments – 1st tier

Input data required:
Contact LD$_{50}$ (μg/bee)

Trigger value:
Toxicity Category
LD$_{50}$ <2 μg/bee = highly toxic
LD$_{50}$ 2 – 11 μg/bee = moderately toxic
LD$_{50}$ >11 μg/bee = practically nontoxic

LD$_{50}$ > 11 μg/bee → low risk; no further studies required
LD$_{50}$ < 11 μg/bee → foliar residue study may be required (lethality of aged residues on foliage when exposed to or ingested by bees)

Validation of defaults and trigger value:
Unknown

Possibility for use for pesticide risk assessment of non-*Apis mellifera* bees:
Toxicity data
• LD$_{50}$s of non-*Apis mellifera* bees; or

• Extrapolation factor for difference in LD$_{50}$ values between *Apis mellifera* and other bees based on toxicity test data. (e.g. see Thompson and Hunt (1999), and van der Steen et al. (2009), for bumblebees)

Trigger value for HQ
• Apply honey bee hazard categories unchanged
• Correct honey bee hazard categories by body weight

References


3. Australian Pesticides and Veterinary Medicines Authority (APVMA)

**Principle**

Tiered approach to assessment of risk from sprayed treatments to honeybees.

**Assessments: Sprayed treatments – 1st tier**

*Calculation of Risk Quotient:*

\[ RQ = \frac{\text{application rate (μg a.i./cm}^2\text{)}}{\text{(acute or oral) LD}_{50}\text{ (μg a.i./bee)}} \]

**Assumption:**

Surface area of a honey bee is 1 cm²

**Input data required:**

Application rate; LD₅₀

**Trigger value:**

Not mentioned (assumed to be 1?)

**Validation of trigger value:**

Not specified

**Possibility for use for pesticide risk assessment of non-*Apis mellifera* bees:**

- Toxicity data
  - LD₅₀ of non-*Apis mellifera* bees; or
  - Extrapolation factor for difference in LD₅₀ values between *Apis mellifera* and other bees based on toxicity test data. (e.g. see Thompson and Hunt (1999), and van der Steen et al. (2009), for bumblebees)

*Exposure data*

- Application rate can be maintained

**Trigger value for HQ**

- Not made explicit by APVMA or EPHC

**References**


B. Other published approaches

1. Honey bee mortality predictor

**Principle**

Calculate expected bee kill (%) given the field application rate, the LD₅₀ of the pesticide to honey bees, and the slope of the probit regression.

**Assessment**
Use either a nomogram or an Excel spreadsheet to calculate expected % kill.

**Input data required**
- Application rate; LD<sub>50</sub>, slope of probit regression

**Assumption**
LD<sub>50</sub> of 1 μg/bee corresponds with 50% mortality at 1 lb/acre, when pesticide is applied as spray to aerial portions of the crop.

**Validation**
Not specified

**Possibility for use for pesticide risk assessment of non-**<i>Apis mellifera</i>** bees:**
- Toxicity data
  - LD<sub>50</sub>s of non-<i>Apis mellifera</i> bees; or
  - Extrapolation factor for difference in LD<sub>50</sub> values between <i>Apis mellifera</i> and other bees based on toxicity test data. (e.g. see Thompson and Hunt (1999), and van der Steen <i>et al.</i> (2009), for bumblebees)

**Exposure data**
- Application rate can be maintained

**References**
- Atkins EL, Kellum D & Atkins KW (1981) Reducing pesticide hazards to honey bees: Mortality prediction techniques and integrated management systems. Leaflet 2883. Division of Agricultural Sciences, University of California, CA, USA.

### 2. Insecticide program risk (IPR) index

**Principle**
Calculation of risk index (IPR) based on honeybee LD<sub>50</sub> and quantity of a.i. applied, for all pesticides applied during a growing season. Refinements of IPR by including weighting factors for time of application or for residual activity of the pesticide.

**Assessment**

\[
\text{IPR} = \sum \frac{kg \ a.i./ha}{\text{honeybee } LD_{50}}
\]

\[
\text{IPR}_t = \sum \left( \frac{kg \ a.i./ha}{\text{honeybee } LD_{50}} \right) \times \text{time of application}
\]
$IPR_t = \sum \frac{(kg \text{ a.e./ha}) \times (\text{residual activity})}{\text{honey bee LD}_{50}}$

with:
Time of application = 1, 2 or 3 (depending on abundance of bees during the season)
Residual activity = 0.1 – 1 (relative value, based on expert opinion)

Input data required:
Application rate; LD$_{50}$; time of application; relative residual activity

Validation:
Comparison of bee communities in 13 commercially managed blueberry fields and 2 minimal chemical/mechanical input fields. Wild bee abundance, species richness and diversity assessed as function of IPR values calculated for the previous year. IPR$_t$ and IPR$_r$ were generally better predictors of abundance, richness and diversity. Overall, explanatory value of the IPR was low (max. $R^2 = 0.52$ of IPR$_t$ for richness)

Possibility for use for pesticide risk assessment of non-**Apis mellifera** bees:
- System was developed for wild bees.
- Use for comparative risk assessment among pest control programmes, and for trends over time. Not easily applicable for individual risk assessments (no critical values for IPR).
- Note: weighting factors are very subjective.
- Note: IPR$_t$ validation is “autocorrelative” (i.e. bee abundance is included in the dependent and the independent variable of the regression).

References

3. PACRAT
Pollinators and pollination in response to Agro-Chemicals and land-use as a Risk Assessment Tool (PACRAT) (part of the EU-ALARM project)

Principle
- spatially explicit emission scenario (using a georeferenced database, calculating a weighted average pesticide concentrations on uncultivated vegetation due to drift in a given surface area);
- fate scenario based on volatilization, wash-off and photodegradation (but not clear how exactly calculated);
• exposure scenario: calculation of total daily intake (TDI) based on dietary uptake (4.3 mg pollen per bee per day) and contact (5 cm² daily contact area) and concentrations on plant tissue;

• effect assessment: LD₅₀ honey bee

leading to risk characterization defined as Toxic Units of the mixture of pesticides applied:

\[ TU_{mix} = \sum_{i=1}^{n} \frac{TU_i}{LD_{50,i}} \]

**Assessment**

Series of equations (see paper) for emission, fate, exposure, effects and risk characterization. [Note: paper does not allow the complete calculation to be repeated, because of gaps in the description of the models].

**Input data:**
GIS with sizes, location and perimeters of fields; cropping data, leaf area indexes; pesticide application rates; pesticide phys-chem. properties; LD₅₀ values.

**Validation:**
Exposure estimation has been successfully validated (Barmaz 2009) (Note: this is PhD thesis; not reviewed yet)

**Possibility for use for pesticide risk assessment of non-** _**Apis mellifera**_ bees:
• Developed for pollinators in general, but so far applied for honey bees only.
• Intended for spatial temporal trends of risk.
• TU peak values generate “aggregated risk”, but not clear how this can be used for risk assessment of individual pesticide (no critical values for TUₘᵢₓ)

**References**

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4. **Population effects of insect growth regulators**

**Principle**
Pre-determined effects due to IGRs are entered in an existing honey bee population model: increased brood mortality; shortened life-span of adult bees; reduced nursing time (precocious foraging).

Possible for assessment of pesticide risks to non-Apis mellifera bees:
- Only possible if sufficient population dynamics information is available for non-Apis mellifera bees to be able to carry out valid population modelling.
- Need brood toxicity and sub-lethal toxicity data on non-Apis mellifera bees.

References


5. Population effects of pesticides (PC BEEPOP)

Principle
Lethal and sublethal effects of pesticides are modelled, using mortality and longevity as inputs (from the BEETOX toxicology database).

[Note: I’m not sure whether the model and the database are still available.]

Possible for assessment of pesticide risks to non-Apis mellifera bees:
- Only possible if sufficient population dynamics information is available for non-Apis mellifera bees to be able to carry out valid population modelling.
- Need mortality and longevity data on non-Apis mellifera bees.

References


6. Risk assessment from pesticide-exposed pollen

Principle
Establishment of classes of predicted environmental concentrations (“PEC classes”) of pesticides in pollen, based on octanol-air partition coefficients and time-weighted average application rates of the pesticide.

Assessment
Calculation of TER for adults and larvae based on PEC and LD₅₀ for honey bees. PEC is calculated on the basis of the PEC-class value for residues multiplied by the daily amount of pollen consumed (dietary exposure – adults and larvae) and collected (contact exposure - adults) by honey bees.

Validation:
No formal validation of the method; but the "calibration" of the PEC classes was done on the basis of 5 pollen residue measurements for 2 pesticides (Note: rather shaky).

Possibility for assessment of pesticide risks to non-Apis mellifera bees:
While the idea on how to predict pollen residues on the basis of pesticide properties is interesting, the method is not sufficiently validated as yet.

References

C. Other possible approaches – not applied yet

1. Pellston workshop suggestion

   Principle

   Tiered risk assessment, based on the principles of the EPPO risk assessment schemes (see A.1), in which non-Apis bees are explicitly included.

   Use of non-target arthropod (NTA) data as surrogate for non-Apis bees: if risk to NTAs is acceptable, risk to non-Apis bees is also acceptable.

   See flow charts in report for further details.

   Note: as is shown below, the executive summary of Pellston, available so far, is not clear on many of the steps. The workshop book chapter should provide more details.

Assessment – foliar treatments

Proposed steps (adults):
1. Assess HQ for honeybee; if HQ < trigger (low risk), then step 2; otherwise, higher tier or risk management.
2. if HQ honeybee < 0.1 of trigger (very low risk): consider low risk to non-Apis bees too; otherwise step 3:
3. Assess HQ for non-Apis bees, using non-target organism data as surrogate (i.e. *Aphidius* & *Typhlodromus*). If HQ > trigger (present risk) go to step 4
   [Note: It is not clear from the report how this should be done. NTA tox tests are lethal rates on glass plates; also which trigger to use in such a case? The NTA trigger or the bee trigger?]
4. Establish oral and contact LD_{50} for relevant non-	extit{Apis} bee species; calculate HQ; if HQ < trigger: assume minimal risk.

**Proposed steps (larvae):**
1. Assess TER for honeybee; if TER > trigger (low risk), then step 2; otherwise, higher tier or risk management.
2. Assess impacts on non-	extit{Apis} bee larvae using 	extit{Apis} larvae test endpoint as surrogate. Is TER < trigger (present risk), then step 3
   [Note: not sure how this is different from step 1?]
3. Establish larval NOAEL for non-	extit{Apis} bee species. If TER > trigger: low risk; otherwise, higher tier or risk management.

**Assessment – soil and seed applied systemic pesticides**

**Proposed steps (adults):**
1. Assess TER for honeybee; if TER < trigger (present risk), then option for 10-d adult honeybee test, or otherwise higher tier testing or risk management. If TER > trigger (low risk) then step 2
2. if HQ honeybee < 0.1 of trigger (very low risk): consider low risk to non-	extit{Apis} bees too; otherwise step 3:
   [Note: which HQ is this, as for systemic pesticides no HQ calculation is being done?]
3. Assess HQ for non-	extit{Apis} bees, using non-target organism data as surrogate (i.e. 	extit{Aphidius} & 	extit{Typhlodromus}). If HQ > trigger (present risk) go to step 4
   [Note: It is not clear from the report how this should be done! NTA tox tests are lethal rates on glass plates, not oral exposure tests; also which trigger to use in such a case?]
4. Establish oral and contact LD_{50} for relevant non-	extit{Apis} bee species; calculate TER; if TER > trigger: assume minimal risk; otherwise higher tier testing or risk management.

**Logical steps (larvae):**
1. Assess TER for honeybee; if TER > trigger (low risk), the step 2; otherwise, higher tier or risk management.
2. Assess impacts on non-	extit{Apis} bee larvae using 	extit{Apis} larvae test endpoint as surrogate. Is TER < trigger (present risk), then step 3
   [Note: not sure how this is different from step 1?]
3. Establish larval NOAEL for non-	extit{Apis} bee species. If TER > trigger: low risk; otherwise, higher tier or risk management.

**Possibility for assessment of pesticide risks to non-	extit{Apis mellifera} bees:**
Risk assessment for non-	extit{Apis} bees is explicitly included in the flow charts, but the exact steps and evaluations to be done are not clear yet. Its suitability for non-	extit{Apis} bees can therefore not yet be assessed.

**References**

2. SETAC-ESCORT risk assessment procedure for non-target arthropods

Principle

Hazard Quotient approach for in-field and off-field exposure of NTAs, using LR50 values (lethal rate) as the toxicity endpoint.

Assessment

\[
In-field\ HQ = \frac{Application\ rate \times MAF}{LR_{50}}
\]

and

\[
Off-field\ HQ = \frac{Application\ rate \times drift\ factor}{vegetation\ distribution\ factor \times LR_{50}}
\]

With:
- MAF = multiple application factor
- LR50 = lethal rate (g a.i./ha) [Note: glass plate exposure tests for a minimum of two species of NTAs]

Trigger value: RQ ≤ 2: low risk.
[Note: trigger used in EU is based on a pesticide industry dataset, which has not been made public!]

Input data:
Application rate; DT50 on vegetation; LR50; drift factor (crop dependent); vegetation distribution factor (default=10)

Validation:
No formal validation of the risk assessment approach has been carried out.

Possibility for use for pesticide risk assessment of non-Apis mellifera bees:

- It may be possible to amend this approach for non-Apis mellifera bees. This would require, however, a modification in the estimate of exposure, so it can be compared to contact toxicity and oral toxicity values which are both expressed as μg/bee

- An example of such an adaptation is the risk assessment procedure developed by Alterra and ICAMA for silkworm in China:

If the pesticide is applied only once during the growing season, calculate the estimated theoretical exposure after a single application (ETEsa) using Formula 5.2.

\[
ETE_{\text{sa}} = AR \times RUD_{95} \times DF_{\text{phi}}, \quad \text{Formula 5.2}
\]

\[
DF_{\text{phi}} = e^\left(\frac{0.693 \times pH1}{DT50}\right), \quad \text{Formula 5.3}
\]

with:
- ETEsa = Pesticide residue level on mulberry leaves in mg a.i./kg fresh weight for single
AR = Application rate of the pesticide (kg a.i./ha).
RUD_{95} = 95th percentile of the Residue Unit Dose for mulberry-type plants or trees. For the Tier 1 risk assessment, the RUD_{95} is set at 950 (mg residue/kg fresh weight of vegetation per kg a.i. of applied pesticide)
DF_{phi} = Degradation factor, when a pre-harvest interval between pesticide application and harvesting of mulberry leaves is applied
DT_{50} = Half-life of the pesticide on vegetation (days)
PHI = Pre-harvest interval (days)

References
