REPORT OF THE WORKSHOP IN BRAZIL

25 – 29 October 2010
Center of Social Insects Study, Universidade Estadual Paulista – UNESP – Rio Claro, SP, Brazil

Participants
J. van der Steen[1], I. Roessink[2], R. Nocelli[3], O. Malaspina[4], M. Kasina[5], P. Kipyab[5], S. Carvalho[4], A. Pereira[4], T. Roat[4], P, Cintra - Socolowski[4], N. van Heesch[1]

[1] Plant Research International, Wageningen, the Netherlands
[2] Alterra, Wageningen, the Netherlands
[3] Federal University of Sao Carlos, Araras SP Brazil
[4] Center of Social Insects Study Universidade Estadual Paulista – UNESP – Rio Claro, SP, Brazil
[5] Kenya Agricultural Research Institute-NARL, Nairobi, Kenya

Introduction
The legislation of pesticides in Brazil and Kenya is based on the risk assessment of pesticides of the European honeybee (*Apis mellifera mellifera*). However, as insect pollination in Brazil and Kenya depends on respectively the Africanized honeybee and *Apis mellifera scutellata* and many species of social, solitary and stingless bees, it can be questioned if *Apis mellifera mellifera* is the most representative pollinator insect on which risk assessment in Brazil and Kenya is based. In other words, are the pollinating insects in Brazil and Kenya adequately protected against the use of pesticides? Therefore, pollinating insects from the Netherlands, Brazil and Kenya are tested in a first tier toxicity test: the acute contact LD_{50}, using the international accepted toxic standard for bee studies, Dimethoate. The result of the test will be combined in a Species Sensitivity Distribution (SSD) showing which species are more or less sensitive to Dimethoate compared to *Apis mellifera mellifera*, providing arguments for an adequate protection of the pollinating bees in Brazil and Kenya.
In the workshop on Knowledge Management of Pesticide Risks to Wild Pollinators for Sustainable Production of High-Value Crops in Brazil and Kenya of 17 – 20 May 2010 in Wageningen it was decided to develop a pesticide toxicity test for key pollinator species. It was decided to develop and perform a first tier study: acute contact LD_{50} of Dimethoate 40%.
The contact LD_{50} test was chosen because is can be applied on all pollinating insects, relevant in this project. Dimethoate was chosen because it is the internationally accepted toxic standard of the first tier studies with honeybees.

Objective
The objectives of the workshop of 25 – 29 October in Rio Claro (Brazil) were

1. to cooperate with the participants from Brazil, Kenya and the Netherlands, involved in this toxicity tests in order to share experience and to agree upon practical performance;
2. to perform preliminary tests to determine the best way of narcotizing and housing of the bees;
3. to perform contact LD_{50} tests with Dimethoate 40% with two Brazilian bee species for validation of the protocol;
4. to agree on the test protocol to determine the acute contact LD_{50} for pollinating insects.

Description of the activities
In order to minimize the variation of the tests, to be performed in Brazil, Kenya and the Netherlands, preliminary work has been performed. The test cages and administration equipment were bought in the Netherlands and transported to Brazil and handed over to the Brazilian and

Kenyan colleagues. The test substance Dimethoate in Brazil was similar to the one to be used in Kenya and the Netherlands. Additionally a sample will be sent to Alterra to verify this.
In the laboratory of the Center of Social Insects Study, Universidade Estadual Paulista – UNESP – Rio Claro, SP, Brazil, the preliminary and two final tests were performed.
The best praxis of handling and housing of the Africanized honeybee and *Scaptotrigona postica,* a stingless bee was determined.
These two bee species were subsequently used for the acute contact toxicity test. The complete test comprising preparation of the Dimethoate concentration range, collection of the bees, narcotization, housing, feeding, administration of the test substance, the observations, data collection and data analysis were done by all participants to share the experience / develop expertise and to agree upon practical experience.
The draft protocol was discussed thoroughly and this resulted in the final study protocol that will be applied in the acute LD_{50} tests in Brazil, Kenya and the Netherlands. The discussion was very fruitful as all workshop members participated intensively. All aspects from collection, housing, design of replicated, determination of the mean weight, how to deal with solitary bees and replicated and statistical analysis were discussed and agreed upon. For some aspects professional statisticians will be asked to verify methods described in the protocol.
The protocol is part of this report.

Results

1. Study protocol to determine the acute contact toxicity of Dimethoate 40% for wild pollinators
2. contact LD_{50} values of
    a. Africanized honeybee
    b. Scaptotrigona postica
    c. Apis mellifera mellifera
    d. Bombus terrestris
3. preliminary SSD based on the LD_{50} values presented in table 1.

Table 1. LD 50 values of pollinating insects of Dimethoate 40%

Species	LD50 µg / bee	Lower limit µg / bee	Upper limit µg / bee
*Scaptotrigona postica**	0.087	0.068	0.111
Scaptotrigona postica	0.123	0.072	0.210
Africanized honey bee	0.223	0.179	0.277
Apis mellifera mellifera	0.2	0.172	0.233
Bombus terrestris	29.717	24.631	35.852

* preliminary test

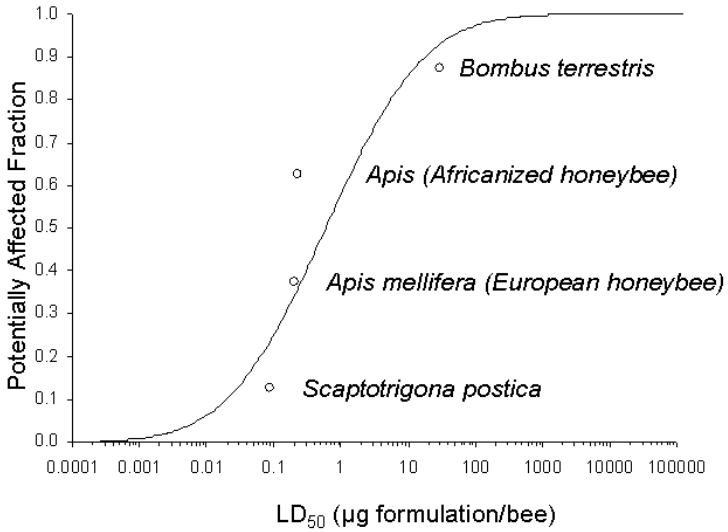

Fig 1. SSD based on 4 bee species.
The LD_{50} values are the actual LD50 values, the x axis is a log scale.

Recommendations
By performing these studies, first steps are made into a new approach in wild pollinator risk assessment. Based upon the application of pesticides, pollinating species in the high value crops and the biology of the pollinating insects, a further risk assessment must be designed.
The recommended steps to be taken are:

1. determination of the contact LD_{50} of Dimethoate 40% for testing of the selected pollinating insects;
2. determination the contact toxicity of pesticides applied in the selected high value crops;
3. design a mechanistic risk assessment procedure containing actual exposure, pollinator biology and agricultural practices to determine the hazard / risk of wild pollinators.