The risk assessment of stressors in bees: a multi-level approach

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Presentation Outline

• The strengths and limitations of laboratory and field studies in relation to their use in the environmental risk assessment scheme.

• The overview of the new test protocol candidates in the risk assessment procedures.

• The extrapolation of the effects from individual to colony/population levels.
The risk assessment of stressors in bees: levels of investigation

Structural levels of a bee’s organization

- Molecular level
- Cellular level
- Tissue level
- Individual level
- Colony (in social bees)/population level

Behavioural and physiological effects

Ecological effects on colony size and survival/population dynamic.
Tiered approach in the registration process of Plant Protection Products: from laboratory to field tests

**Laboratory tests**
- Effects on individual bees;
- Individual exposure;
- 100% of exposure level (protection of the compound by degradation);
- Controlled conditions;
- Many replicates;
- Lower cost;

**Field tests**
- Effects on colony;
- Colony exposure;
- Field level of exposure (real exposure);
- Higher cost

+ conservative

+ realism
Establishment of a WG with experts in the area of bees health and exposure (September 2011)

EFSA Opinion on the science behind... ...adopted in April 2012

Draft Guidance (1° round of public consultation) in September 2012
### Laboratory tests

#### Source of variations of the LD50

(i.e. Imidacloprid: 4-600 ng/bees)

#### Test temperature

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>Fipronil</th>
<th>Clothianidin</th>
<th>Thiamethoxam</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 0.5</td>
<td>11.72</td>
<td>0.90</td>
<td>1.59</td>
</tr>
<tr>
<td>30 0.5</td>
<td>5.76</td>
<td>1.45</td>
<td>2.65</td>
</tr>
<tr>
<td>35 0.5</td>
<td>2.54</td>
<td>1.45</td>
<td>3.62</td>
</tr>
</tbody>
</table>

| N     | 4   | 4   | 3   |

- Medrzycki et al. (2012) *Julius-Kühn-Archiv*

#### Bee age

- Young bee
- Foragers

- Medrzycki et al. (2012) *Julius-Kühn-Archiv*
### Laboratory tests

#### Source of variations of the LD50
(i.e. Imidacloprid: 4-600 ng/bees)

#### Nutritional status

<table>
<thead>
<tr>
<th>Pollen Diet</th>
<th>24h</th>
<th>48h</th>
<th>72h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD50</td>
<td>SD</td>
<td>LD50</td>
</tr>
<tr>
<td>MIX</td>
<td>4.730</td>
<td>0.156</td>
<td>2.569</td>
</tr>
<tr>
<td>MAIZE</td>
<td>3.643</td>
<td>0.459</td>
<td>1.855</td>
</tr>
<tr>
<td>p (t Stud)</td>
<td>0.018</td>
<td></td>
<td>0.015</td>
</tr>
</tbody>
</table>

Tosi et al. (2013) *Am. Bee Jour.*

#### Health status

Field tests

Multi-exposure routes

Source of variations in field studies

Multi-compound exposure

EFSA PPR (2012). EFSA Journal

CRA-API (2009, 2010). Apenet project

Bees (N=93)

- 1 compound: 14%
- >1 compounds: 86%

Pollen (N=149)

- 1 compound: 16%
- >1 compounds: 84%

Wax (N=296)

- 1 compound: 32%
- >1 compounds: 68%
Field tests

Sanitary status of the colonies

Source of variations in field studies

Environmental diversity (at landscape level)
Field tests

Colony size and strength

Source of variations in field studies

Meteorological conditions/Season

Other source of variations: genetic, local climatic conditions…

Dataset: ApiPop.res

Total number of adult bees

Simulation time

Total number of adult bees

0 50 100 150 200 250 300 350 400

0 5000 10000 15000 20000 25000 30000
Field tests

Size of the treated field

1.5 Km

Foraging area

700 ha

1 ha
Field tests

Distance hive-treated field

Several bees exposed at 1.34 ng of thiametoxam did not return to the hive when they were released at 1 Km from the hive.

Henry et al. (2012). Science
Field tests

Statistical power of the test

No real effects in field or low statistical power?

Cresswell (2011). *Ecotoxicology*
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Overview of the new test methods

*In vitro* larvae test

Aupinel et al. (2005)
*B. Insectology*

Test the effects of dust in cage and in field

Sgolastra et al. (2012).
*B. Insectology*

Georgiadis et al. (2012).
*Julius-Kühn-Archiv*
Overview of the new test methods

Method to feed individual bees

Test methods on solitary bees

Cage or field studies to assess the nesting activity and fecundity in nesting females of solitary bees

Bioassay in laboratory with larvae of *Osmia*

Ladurner et al. (2003). *Apidologie*

Konrad et al. (2008). *Plos One*

Sgolastra et al., unpublished

Ladurner et al. (2008). *J. Econ. Entom.*
Overview of the new test methods

Laboratory based *Bombus* micro-colonies for evaluating reproductive effects

Test methods on bumblebees

Combined pesticide exposure by oral and contact

Effects on queen production in colony exposed in the lab and development in field

Mommaerts et al. (2010). *Ecotoxicology*

Gill et al. (2012). *Nature*

Whitehorn et al. (2012). *Science*
Overview of the new test methods

Effects on thermoregulation

Test methods on sub-lethal effects (physiological endpoints)

- Stabentheiner et al. (2010). *Plos One*

Effects on HPG development

- Hatjina et al. (2012). *Apidologie*
Overview of the new test methods

Effects on learning capacity
(beroingal endpoints)

Test methods on sub-
lethal effects

Effects on homing ability

Decourtye et al. (2005).
Arch. Env. Con. Tox.
CRA-API (2009, 2010)
Apenet project

Bortolotti et al. (2003). B. Insectology;
Schneider et al. (2012). Plos One;
Henry et al. (2012). Nature
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Effects from individual to colony
Effects on homing ability: different scenarios

Sublethal intoxication by pesticide

Forager disorientation

- A limited number of bees involved ➔ Colony survival
- Medium number of bees involved ➔ Colony weakening
- A lot of bees involved ➔ Colony loss

Colony weakening

- No other stressors involved
- Other stressors involved
Effects from individual to colony

Effects on homing ability: different scenarios

Sublethal intoxication by pesticide

Forager disorientation

Colony weakening

Colony loss

How many bees (definition of the thresholds)?
Can models help?
Which variables/parameters need to be considered in the model and which values need to be assigned to the parameters (egg laying, background mortality, etc.)?

A lot of bees involved

Medium number of bees involved

A limited number of bees involved

Other stressors involved

How many bees (definition of the thresholds)?
Can models help?
Which variables/parameters need to be considered in the model and which values need to be assigned to the parameters (egg laying, background mortality, etc.)?
Effects from individual to colony

“Models are always wrong…but many of them are useful”

How a wrong model can give a correct answer?
In the same way as old maps were useful for travelers in the past

Marco Polo
Effects from individual to colony
Effects on homing ability: different scenarios

Sublethal intoxication by pesticide

Forager disorientation

- A limited number of bees involved
- Medium number of bees involved
- A lot of bees involved

Colony weakening

Other stressors involved

Colony loss

Which other factors need to be considered in the regulatory process (varroa, nosema, virus, food quality, management)? How to include them?
The risk assessment of stressors in bee: conclusions

Difficult to extrapolate the effects from individual to colony due to the increasing of the complexity of the system.

- Capacity to describe the mechanistic basis of the effects
- Capacity to describe the dynamical properties of the abiotic and biotic interactions

Level: Molecular/cellular level | Individual level | Colony/population level
Thank you for your attention
Cited references


CRA-API, 2009. “Effects of coated maize seed on honey bees”. Report based on results obtained from the first year of activity of the APENET project. 30 pp. http://www.reterurale.it/flex/cm/pages/ServeAttachment.php/L/IT/D/5%252Ff%252Fc%252FD.5d70d88c74b5011d07e8/P/BLOB%3AID%3D4600


