

What about Lepidoptera? Future requirements and proceedings on risk assessment towards Non Target Arthropods

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Introduction

Ever since the European Food Safety Authority (EFSA) firstly published the “Scientific Opinion addressing the state of science of risk assessment on plant protection products for non-target-arthropods” (EFSA, 2015), a number of challenges and necessary improvements concerning the risk assessment and study design have been raised for discussion. One aspect among numerous newly raised concerns comes with including a new group of organisms to risk assessment, the large and important group of Lepidoptera species. The EFSA working group “considers Lepidoptera to be important drivers for the ecosystem services pollination and food web support”, but yet validated and reliable testing protocols for oral and contact exposure are missing, not even speaking of harmonized and accomplished official ring tests on broad international scale.

Requirement Source:



An important group for which standard test protocols are missing is Lepidoptera. The working group considers Lepidoptera to be important drivers for the ecosystem services pollination and food web support (see section 4). For Lepidoptera larvae, there are test guidelines available in the open literature that could be adapted for oral and contact exposure (see Appendix G). Grasshoppers, plant hoppers and several groups of flies and wasps are also missing from the standard test systems.

Applying the selection criteria for tier 1 test systems (take in to account spread over taxonomic groups, toxicologically sensitive indicators, sensitive life stages/developmental processes, relevant exposure routes) to Appendix G, based on the test systems that are currently available or could be practicable in the near future, the working group recommends available tests with the following species to be carried out already at tier 1:

<i>Aphidius rhopalosiph</i>	leaf-dwelling parasitoid (Hymenoptera)
<i>Typhlodromus pyri</i>	leaf-dwelling predator (Acari)
<i>Coccinella septempunctata</i>	mobile leaf-dwelling predator (Coleoptera)
Lepidopteran larvae	leaf-dwelling herbivore (Lepidoptera)



Figure 1: Imago of *Pieris brassica*

Technical Aspects

A few methodologies for Lepidoptera testing exist and are also mentioned by EFSA (Appendix G of the scientific opinion), but these are either limited to special product use (e.g. *Bacillus thuringiensis* strains), or not yet developed to high level end use (e.g. *Pieris brassica*, Figure 1). In Appendix G, the mentioned setup for *Pieris brassica* seems to be a promising approach, although it may be limited to oral exposure. A good and feasible approach for testing oral exposure routes on Lepidopteran larvae would be the *Bombyx mori* setup as described by guideline published by the Chinese Ministry of Agriculture (ICAMA, see also Table 1 and Figure 2 to 4).

Table 1: Framework characteristics of a Lepidoptera test setup

Parameters	<i>Bombyx testing</i>
Study design	- Limit test / full study consisting of seven to nine treatment groups (control, solvent control and five to seven test item concentrations)
Replication	- Three replicates (=test units) per treatment, ten larvae (2 nd instar larvae) per replicate (i.e. 30 larvae per treatment)
Treatment	- Treated leaves are prepared once at the beginning of the study and stored in a refrigerator until required - Test organisms used for the test are within 24 hours of hatching (2 nd instar larvae) - Two mulberry leaves per treatment are dipped into the corresponding application solution for approx. five sec and drying within a fume hood before test start - Fresh strips of treated leaves (approx. the same width as the silkworm body length) are provided daily, and any leaves remaining from previous day are removed
Exposure	Up to 120 hours within a petri-dish (Figure 2)
Endpoint	Mortality of larvae calculated for each treatment, corrected by the mortality of the control / solvent control

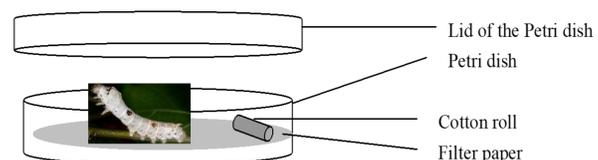


Figure 2: Test vessels for oral exposure setup on Lepidopteran larval species



Figure 3: Larvae of *Bombyx mori*



Figure 4: Imago of *Bombyx mori*

Outcome & Future Perspectives

As for methodology, in all so far conducted tests on *Bombyx mori* in our Laboratory (see also an example in Figure 5), the control mortality was below 10 % and therefore fulfilled the main guideline criterion (i.e. $\leq 10\%$ mortality in the control). This indicates a feasible and reproducible setup with high possibility of transfer and implementation for other Lepidopteran species.

Towards a possible revised risk assessment and the EFSA recommendations it remains rather inconsistent that *Pieris brassica* is classified as pest organism to some farming cultures (e.g. cabbage), an aspect which counteracts to the overall combined approach of plant protection products and use of “beneficials”. Further on, there are a lot of species from the Lepidopteran group (e.g. diamondback moths, cabbage loopers, tip moths) required for efficacy testing throughout registration process of plant protection products. By showing efficacy of the respective plant protection product on Lepidoptera species, it may ultimately counteract towards environmental testing at a later stage then, finally failing EITHER efficacy (= useless product) OR ecotoxicological (= product too harmful to organisms) requirements. Lastly, the here presented setup is limited to oral exposure and animals at larval stages, whereas full grown adults, contact exposure as well as, subsequently, reproduction endpoints remain uncovered yet.

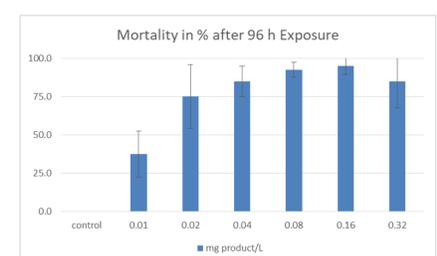


Figure 5: Test results *Bombyx mori* after 96 hours

Conclusion:

- Experience and accomplished work reveals a possible approach for a test method on Lepidoptera species under oral exposure, based on an accepted and reliable Chinese guideline method for Silkworms, *Bombyx mori* (ICAMA, 2014).
- Methods and setups mentioned in Appendix G of the EFSA scientific opinion are counteracting and/or too limited to a smaller group of specialized products and active compounds.
- Requirements and contradictions for such a setup under risk assessment for this specific group of organisms remains unsolved and needs further clarification and research.
- Further research is needed on contact toxicity as well as standardized, comparable and reproducible species.
- How to expose and assess adult representatives within this group of species? What about reproduction endpoints?

Sources

1. ICAMA 2014, Chemical pesticide environmental safety evaluation test rule 2.2.9 P23-25 Guidelines for the testing of chemicals: Bombyx, Acute Toxicity Test.
2. EFSA 2015, European Food Safety Authority, Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods, EFSA Journal 2015;13(2);3996