# Investigation of Key Parameters to Adapt Ecotoxicological Testing to Microbial Pesticides - An Example with a Social Insect and an Aquatic Invertebrate

Florine Ory[1], Magdaléna Cornement[1], Lauren Ruysschaert[1], Helene Eckenstein[1], Stephen Vinall[2], Julie Masse[1] and Stefan Höger[1]

[1] Innovative Environmental Services (IES) Ltd, Benkenstrasse 260, 4108 Witterswil, Switzerland
[2] Mambo-Tox, 2 Venture Road, University Science Park Southampton, SO16 7NP, United Kingdom

SETAC Europe 34th Annual Meeting
Seville, Spain, May 5th-9th, 2024
Poster n° 2.07.P-Tu160

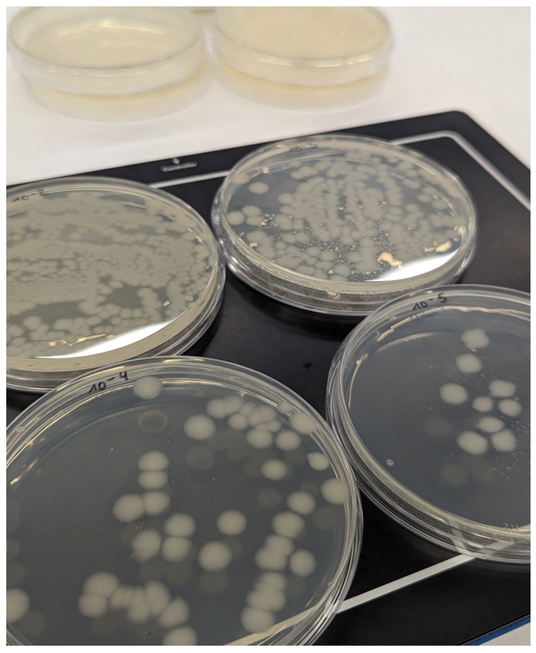
## Introduction

**Biopesticides** operate through biological mechanisms to manage pests, arousing growing interest in their development as sustainable solutions within the global crop protection landscape. In contrast to synthetic chemicals, microbial pesticides are relatively specific and have **targeted modes of action** that include, resources competition, infectivity, and pathogenicity, rather than toxicity alone.

Such mechanisms **pose potential ecological risks** to non-target organisms, necessitating thorough assessments tailored to specific environmental contexts, encompassing exposure routes, developmental stages, and life cycles. Conventional testing protocols, typically designed for assessing chemical toxicity, must undergo adaptation to account for the ecological attributes of the tested organisms.

**Here, the impact of a widely used microbial pesticide, *Bacillus thuringiensis,* was evaluated on honey bee larvae and young daphnids.**

By modifying parameters of the study protocols, we aimed to create a more accurate representation of **microbial exposure scenarios**, thus facilitating the understanding of its ecological implications.

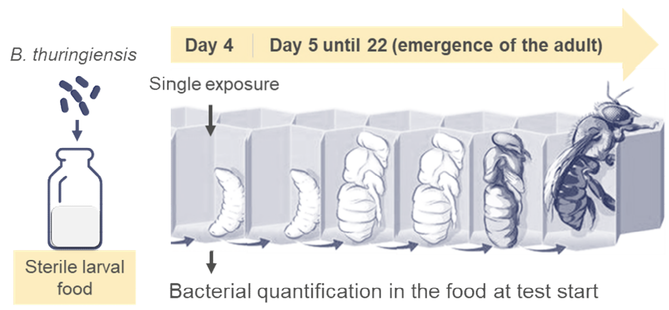


Culture system of *Bacillus thuringiensis aizawai* (left) and *Daphnia magna* (right) at IES.

## Materials & Methods

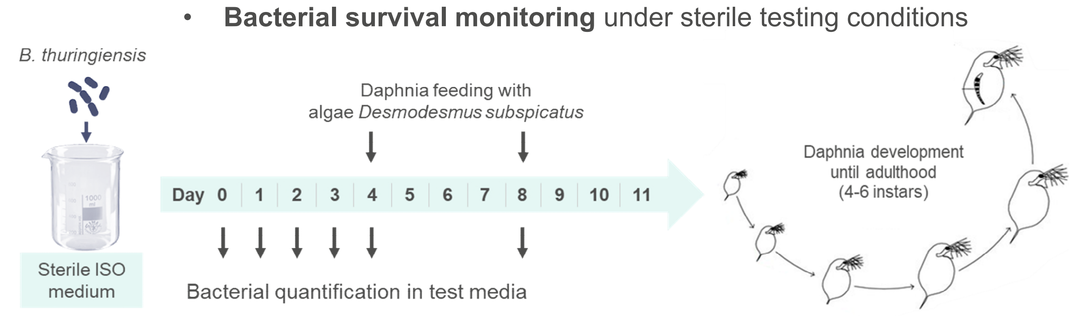
### Social insect – honey bee larvae (*Apis mellifera*):

- Guidelines: Acute (OECD 237) and Chronic Larval Test (OECD GD 239)
- **Single oral** exposure (feeding)
- Mortality monitoring extended to the **emergence of the adult** (22 days)
- **Bacterial dose verification** in the food through **CFU counting**




### Aquatic invertebrate – young daphnids (*Daphnia magna*)

- Guidelines: adapted from Acute Daphnia Toxicity Test (OECD 202)
- **Static** exposure (test medium)
- Mortality/immobility monitoring extended to **adulthood** (11 days)
- **Bacterial dose verification** in the medium through **CFU counting**
- **Bacterial survival monitoring** under sterile testing conditions




## Results

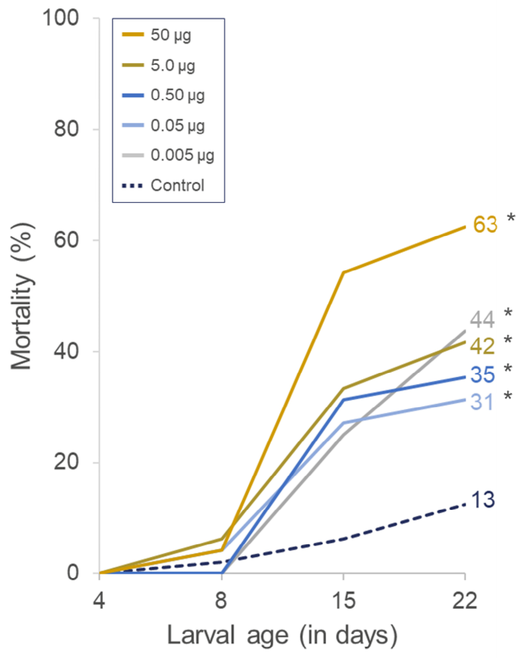



**Figure 1.** Mortality of *in vitro* reared honey bee larvae after single exposure (at day 4) to *B. thuringiensis aizawai* at different doses (µg per larva). Asterisks represent statistically significant differences with the control group (Step-down Rao-Scott-Cochran-Armitage Test; $p < 0.050$; one-sided greater; N=48 larvae per condition, 3 replicates of 16 larvae).

**Table 1.** Summary the bacterial quantification of the inoculated and control food fed to larvae.

| Target doses of *B. thuringiensis* (µg per larva) | Expected CFU/larva | Counted CFU/larva |
|---|---|---|
| Control | 0 | 0 |
| 0.005 | $3.0 \times 10^2$ | $2.5 \times 10^2$ |
| 0.05 | $3.0 \times 10^3$ | $3.3 \times 10^3$ |
| 0.50 | $3.0 \times 10^4$ | $3.0 \times 10^4$ |
| 5.0 | $3.0 \times 10^5$ | $2.7 \times 10^5$ |
| 50 | $3.0 \times 10^6$ | $2.2 \times 10^7$ |

Bacteria were counted from triplicate nutrient agar plates, incubated at 33°C for 24 hours and the average CFU/mL was calculated.

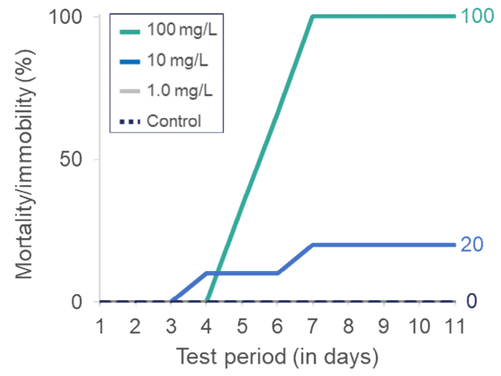



**Figure 2.** Mortality of young daphnids exposed to different concentrations of *B. thuringiensis aizawai* during a period of 11 days. N=10 daphnids per condition, 2 replicates of 5 individuals.

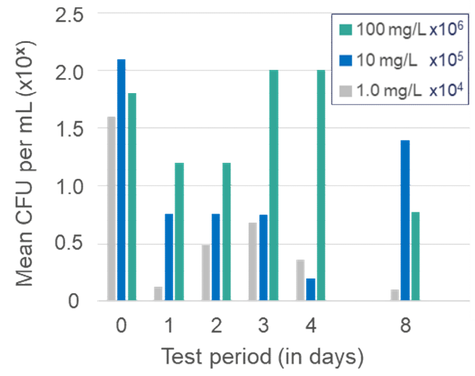
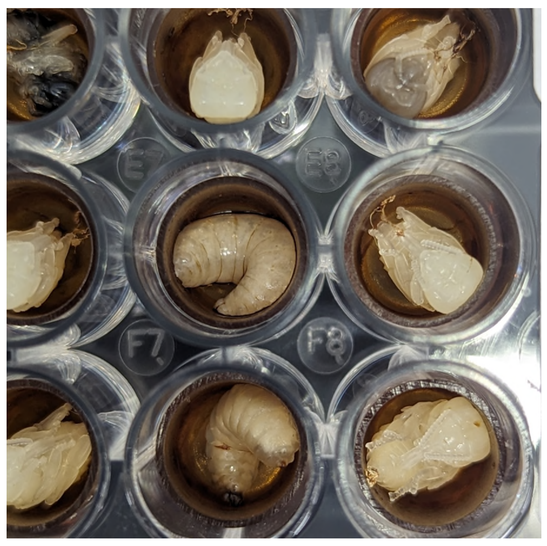



**Figure 3.** Effective colony-forming units (CFU) of *B. thuringiensis aizawai* from inoculated ISO medium at test start and time points of the test period. The average CFU/mL was calculated from triplicate inoculated agar plates.

**Table 2.** Summary of the bacterial quantification of the inoculated and control ISO media during the test period.

| Target doses of *B. thuringiensis* (mg/L) | Expected CFU/mL | Effective CFU/ml | | | | | |
|---|---|---|---|---|---|---|---|
| | | Test start | Day 1 | Day 2 | Day 3 | Day 4 | Day 8 |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1.0 | $6.0 \times 10^4$ | $1.6 \times 10^4$ | $0.1 \times 10^4$ | $0.5 \times 10^4$ | $0.7 \times 10^4$ | $0.4 \times 10^4$ | $0.1 \times 10^4$ |
| 10 | $6.0 \times 10^5$ | $2.1 \times 10^5$ | $0.8 \times 10^5$ | $0.8 \times 10^5$ | $0.8 \times 10^5$ | $0.2 \times 10^5$ | $1.4 \times 10^5$ |
| 100 | $6.0 \times 10^6$ | $1.8 \times 10^6$ | $1.2 \times 10^6$ | $1.2 \times 10^6$ | $2.0 \times 10^6$ | $2.0 \times 10^6$ | $0.8 \times 10^6$ |

Honey bee larvae failing to pupate (left) after the oral exposure to *B. thuringiensis aizawai* (right).



## Discussion and Conclusion

The biopesticide *Bacillus thuringiensis aizawai* **induced mortality** in both tested organisms. The importance of conducting a test period spanning the developmental cycle of test organisms, alongside the selection of a suitable exposure route was demonstrated.

**Honey bee:**

- ✓ Mortality mostly induced during pupation phase (between 8 and 15 days)
- ✓ Dose related response (except one group)
- ✓ Bacterial dosage confirmation from food

**Daphnia**:

- ✓ Mortality induced after 4 days
- ✓ Dose related response
- ✓ Bacterial dosage and survival under test condition were confirmed

If you have any questions, please do not hesitate to contact us.

## References and Acknowledgements

- OECD No. 237, Guideline for testing of chemicals: Honey bee (*Apis mellifera L.)* larval toxicity test, single exposure. 2013.
- OECD No. 239, Guidance document on honey bee larval toxicity test following repeated exposure, Series on Testing and Assessment, 2016.
- OECD No. 202, Guideline for Testing of Chemicals, Daphnia sp., Acute Immobilisation Test, 2004.
- OPPTS 885.4000, Background for Nontarget Organism Testing of Microbial Pest Control Agents, EPA, 1996.

We are thankful to the IES Ecotox team for their support during the performance of the studies and internal trials.