Session: : Biocides and Veterinary Medicines: latest developments in regulatory risk assessment, research and monitoring
Platform Presentation
Session room M, Monday May 14th, 2018, 09.20 a.m., ID: 34

How can mesocosm studies increase realism in risk assessment of biocides and veterinary medicines?

László Dören[1], Udo Hommen[2], Klaus Peter Ebke[3] and Rolf-Alexander Düring[4]

[1]ERM GmbH, Siemensstrasse 9; 63263 Neu-Isenburg, Germany
[2]Fraunhofer IME, Auf dem Aberg 1, 57392 Schmallenberg, Germany
[3]Mesocosm GmbH, Neu-Ulrichstein 5, 35315 Homberg (Ohm), Germany
4 Justus Liebig University Giessen, Heinrich-Buff-Ring 26, 35392 Gießen, Germany
E-mail contact: laszlo.doeren@erm.com

1. Introduction

Mesocosm studies can be used to assess the environmental impact of potential stressors based on model-ecosystems under realistic environmental conditions. They are an important link from laboratory to field. Mesocosms provide the assessment of a broad range of different species of different ecological groups forming food webs with complex interactions. Therefore mesocosm studies can support a better understanding of the environmental impact of stressors on population level as well as on ecosystem level (e.g. direct and indirect effects on community structure and ecosystem functions as primary production). In addition, mesocosm studies provide data on the fate of test substances under realistic outdoor conditions, which can be used to test the prediction based on laboratory studies.

While for the risk assessment of Plant Protection Products (PPP) mesocosm studies are an established higher tier approach and are considered as the surrogate reference tier [1], the use of mesocosm studies for risk assessment of biocides, veterinary medicines and chemicals under REACH is rare, although mesocosms are recommended in the *Guidance on information requirements and chemical safety assessment - Chapter R.10: Characterisation of dose [concentration]-response for environment* [2] and in the *Guidance on the Biocidal Products Regulation - Volume IV Environment - Assessment and Evaluation* [3] both provided by ECHA.

One reason for this might be, that mesocosm studies have the reputation to be very complex and difficult to evaluate by regulators. This presentation intends to take some fears of contact with mesocosms. It will explain the most important aspects to validate the quality of a mesocosm study and the relevance of the results. Further, it will give some insights to the use of (aquatic) mesocosm studies in the context of PPP risk assessment and will provide important aspects for planning a mesocosm study for biocides, veterinary medicines and chemicals in the context of REACH.

2. Planning a mesocosm study

While planning a mesocosm study the following points should be considered:

- **Specific questions to be addressed** should be based on exposure assessment and lower tier tests.
- **Type of mesocosm:** Ponds, ditches, streams or (indoor) microcosms. Each type has its specific advantages and limitations. The choice of type should be based on the specific question to be addressed.
- **Test design:** Preferably 5 or more test concentrations with at least 2 replicates per level should be used. For the controls at least 4, better 5 replicates are recommended (e.g. controls (n=5) + 5 treatment levels (n=2)).
- **Test duration**: Usually at least 8 weeks after first application.
- **Exposure**: Multiple or single application or constant exposure? Worst case scenario covered? Appropriate analytical monitoring of the test substance (example see fig.1)?
- **Endpoints**: Which endpoints (e.g. zooplankton, macroinvertebrates, phytoplankton, periphyton, macrophytes) are assumed to be the most relevant/sensitive? Can secondary effects assessed by the choice of endpoints? Should bioassays introduced into the mesocosms to meet specific questions (e.g. Gammarids)? What sampling intervals are appropriate to cover short-term effects and long-time effects and recovery (example see fig. 2).

3. Preparing a mesocosm study

The set-up of a mesocosm is usually performed with natural sediment and natural or tap water. Analyses in front of the test start have to prove that sediment and water are free from environmental relevant contaminations. Depending on the specific question additional introduction of invertebrates and macrophytes are possible. The establishment period of a mesocosm should be at least a few months before test start to assure a stable ecosystem, when assessing the impact of a test substance. A low variability between the replicates at the start of the test regarding the different biological endpoints and the phys-chem. water parameters is most important to get good results from the statistical point of view. The second important point at this stage is to have a representative biocenosis with a sufficient number of potentially sensitive and vulnerable species with abundances allowing an evaluation of effects.

4. Evaluating a mesocosm study

The evaluation of a mesocosm study for risk assessment of plant protection products according to EFSA (2013) is usually performed using:

- **multivariate statistics** to assess effects on community structure (e.g. Diversity indices, Principal Response Curves);
- **univariate analysis** of population abundances and other endpoints usually based on ANOVA (e.g. using Dunnett or Williams-test);
- **calculation of NOECs** and related **Minimum Detectable Difference (MDD)**;
- **effect classification**.

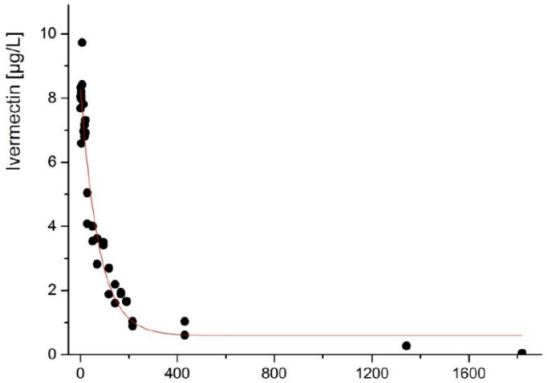

Figure 1: Example: Analysis of ivermectin in a mesocosm study. x-axis: time in hours. y-axis: measured (black dots) and modelled (red line) concentration of ivermectin [µg/L]. HPLC fluorescence detection after derivatisation, according to [4].

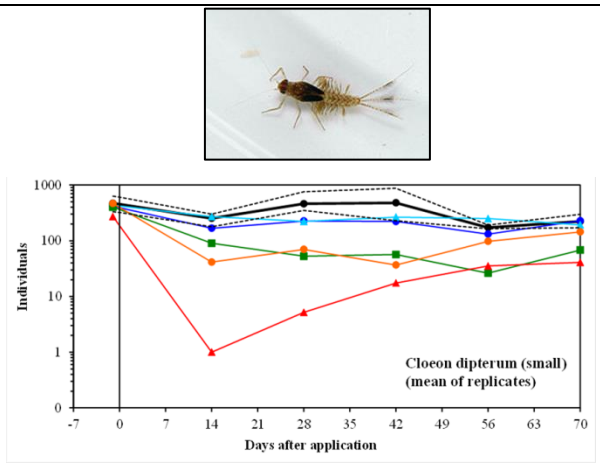

Figure 2: Example: Population-dynamics of Cloeon dipterum in a mesocosm study after single application of biocide. Black line: controls. Red line = highest test concentration [5]

5. References

[1] EFSA. 2013. Guidance on tiered risk assessment for edge-of-field surface water. www.efsa.europa.eu

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[4] Wohde M, Blanckenhorn WU, Floate KD, Lahr J, Lumaret J-P, Römbke J, Scheffczyk A, Tixier T, Düring R-A (2016) Analysis and disipation of the antiparasitic agent ivermectin in cattle dung under different field conditions. *Environmental Toxicology and Chemistry 35: 1924–1933*.

[5] Hommen U 2015. Micro- and mesocosm studies as a higher tier option for aquatic risk assessment of biocides. Presentation at 4th International Fresenius Conference “Environmental Risk Assessment of Biocides”, 21 and 22 October 2015 in Cologne, Germany.