Introduction

- Exposure of aquatic plants to chemical stressors can be temporary, e.g. due to degradation or transport after single or multiple entries.
- Neglecting population recovery in risk assessment is protective but might be over-restrictive resulting in unnecessary losses of crop yields. Thus, risk assessment options include the Ecological Threshold Option (ETO) or the Ecological Recovery Option (ERO) [1].
- The recovery subgroup of the SETAC Plants Interest Group aims to review the different approaches to analyse and predict recovery of plants and to make suggestions how recovery could be included in a risk assessment framework.

Definitions

- We refer to the definition of terms by EFSA in [2] (see Figure 1) for recovery as “the return of the perturbed ecological entity or process (...) to the NOR (Normal Operating Range) observed in the undisturbed state of the ecosystem of concern, for example to a level that is not significantly different from that in control or reference systems”.
- Specific protection goals under ERO allow
  - for algae large effects for days, medium effects for weeks, and small effects for several months
  - for macrophytes medium effects for weeks, and small effects for several months, but no large effects on the abundance and/or biomass of vulnerable populations [1].

Approaches

- Refined exposure tests in the lab (Tier 2C)
  - E.g. pulsed exposure in modified 221 (Lemna) or 239 (Myriophyllum)
  - Guidance [1] recommends use of same endpoint (ErC50) and same assessment factor (i.e. 10) for Tier 2C tests.
  - Tier 2C intended for ETO, but not ERO,
  - only recovery of population growth rate can be assessed, no recovery of biomass / standing stock in community context.
  ⇒ Tier 2C tests with algae or plants do not fit directly to ERO.

- Population and community level experiments, e.g. mesocosm tests (Tier 3)
  - Analysis of effects and recovery under more realistic conditions (e.g. outdoor, community context, indirect effects) on phytoplanктon, periphyton, floating, submersed and emergent macrophytes.
  - In order to monitor biomass or shoot length of rooted plants in the sediment potted plants (bioassays) can be used.
  - However, no full community context and potential growth over the full study. For macrophytes, recovery of abundance / biomass can be difficult to assess in mesocosms.

Conclusions

1. Modified exposure tests in the laboratory only allow to demonstrate reversibility of effects on growth rate but do not allow direct derivation of an ERO-RAC.
2. (Semi-)field tests can address population recovery, but with some methodological limitations for potted plants.
3. Modelling allows to make better use of data and to extrapolate from lab to the field situation. However, information on long-term dynamics in the field and the normal operation range for model verification are missing for focal plant species.

Effect modelling (Tier 2 and Tier 3)

- Tool to predict effects of dynamic exposure patterns and to extrapolate from lab to field (e.g. [3]).
- Use of Tier 1 and Tier 2 data for calibration and verification of the substance related toxicokinetic-toxicodynamic model
- Straight forward modelling of Tier 2C tests for dynamic exposure (e.g. from FOCUS)
  ⇒ ETO-RAC for dynamic exposure.
- Modelling of biomass dynamics in the field requires basic ecological data on how growth depends on environmental factors and long-term control data (to quantify NOR).

Reference

How to consider recovery of aquatic plants in risk assessments?

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1. Introduction

Exposure of non-target plants to plant protection products can be restricted in time if the substances show fast dissipation, e.g. by degradation or transport. In such cases, the plants might recover if the effects are reversible. Neglecting the recovery potential in the risk assessment is definitely protective but might be over-restrictive resulting in unnecessary losses of crop yields. The recovery subgroup of the SETAC Plants Interest Group aims to review the different approaches to analyse recovery of plants and to make suggestions how recovery of these could be included in a risk assessment framework. In this presentation, we will focus on aquatic algae and macrophytes and the regulation of plant protection products in the EU.

2. Definitions

We refer to the definition of terms by EFSA in [1] (see Figure 1). Recovery can be distinguished in actual and potential recovery. Actual recovery refers to ‘the return of the perturbed ecological entity or process (e.g. species composition, population density or ecosystem services) to the NOR (Normal Operating Range) observed in the undisturbed state of the ecosystem of concern, for example to a level that is not significantly different from that in control or reference systems’ (EFSA 2014). As the specific protection goals for algae and macrophytes are defined on the population level (EFSA 2013), recovery should be assessed for population endpoints like population growth rate, abundance or biomass. Experimentally, recovery of algae and macrophytes can be assessed in single species laboratory tests or micro- and mesocosm studies.

Figure 1: Schematic illustration of the effect period and related recovery times (copied from [1]).

3. Approaches

3.1. Laboratory single species tests

The OECD test guidelines with algae (201) and *Lemna* sp. (221) already assess a population level endpoint, i.e. the inhibition of the (population) growth rate. For rooted macrophytes e.g. *Myriophyllum spicatum* in OECD test guideline 239, the growth of shoot length or biomass of individual plants is measured but because vegetative growth is the most important process of population growth of macrophytes, this can be
considered as a surrogate for population level effects on aquatic plants unless sexual reproduction is affected. The OECD standard tests are conducted under constant exposure over the test duration of 4, 7 or 14 days for algae, *Lemna* and *Myriophyllum*, respectively. To address pulsed exposure events as often predicted for edge of field water bodies, so-called refined exposure laboratory toxicity tests (Tier 2C, [2]) can be conducted, e.g. exposing *Lemna* over 1 day followed by growth in control medium over 6 days. Such tests can be used to test on the reversibility of a growth inhibition by comparing growth rates of exposed and control plants. The tests can also be adapted to test multiple pulses. However, as the plants are kept in the exponential growth phase, these studies can only analyse the recovery of the growth rate but not of abundance or biomass. The Aquatic Guidance Document [2] recommends using the same endpoint (ErC50) and the same assessment factor (i.e. 10) for such refined exposure tests with plants. However, it is not clear from the existing guidance how prolonged tests, for example with multiple peaks, should be used in a risk assessment considering recovery.

### 3.2. Microcosm and mesocosm studies

Micro- and mesocosm studies offer the possibility to assess effects and recovery of plants under more realistic conditions, e.g. under field conditions in an (model) ecosystem context. In practice, recovery is considered as acceptable in such studies, if the effect period (significant difference to control, see Figure 1) is restricted to eight weeks [2]. In contrast to the laboratory growth inhibition tests, abundance, biomass (or, for macrophytes, shoot length or frond number) are used as test endpoints. Thus, recovery can only be demonstrated in such studies if the control population shows no permanent exponential growth and thus reaches a plateau or steady state. However, this might be less expected compared to the field situation, as the studies are often set up with potted small and young (shoots of) plants while in the field an established standing stock might be exposed. Experimental challenges include achieving good growth of a representative number of species. Different macrophytes species can be introduced but the development of the algae community is not manageable since algae species can pop up for a short-time only in such a microcosm or mesocosm study.

### 3.3. Modelling

Mechanist effect modelling is considered as tool to extrapolate from lab and semi-field experiments to the situations expected in the field. For example, a substance related part of the model (toxicokinetics and toxicodynamics) is calibrated and tested based on laboratory growth inhibition tests while the ecological part is based on available knowledge of the dependence on growth from different environmental factors like temperature, radiation, nutrient levels and density dependence. Modelling refined exposure tests (Tier 2C) assuming a fixed control growth rates under laboratory conditions is a straightforward application of such models to extrapolate from the few tested exposure profiles to the diversity of the exposure profiles predicted e.g. by the FOCUS models. It is possible to stick very close to the Tier 1 assessment, e.g. by simulating the worst case time window of the exposure profile to calculate the ErC50 and to use the standard assessment factor of 10 to derive the regulatory acceptable concentration. For extrapolation to the field situation (Tier 3), the availability of long-term data sets of seasonal dynamics of plant growth in the field to verify the models is a major challenge at the moment. Another open question is how to use of results of such models for risk assessment. The Aquatic Guidance Document [2] defines specific protection goals for aquatic plants but these are only semi-quantitative. Only ‘negligible’ effects are considered acceptable under the ecological recovery option while for the ecological recovery option, ‘small effects’ over ‘months’ and ‘medium effects’ over ‘weeks’ are considered acceptable. To define acceptable magnitude and duration of effects on field populations, we could use the pragmatic criteria used for evaluation of mesocosm studies [2].

### 4. Conclusions

Modified exposure tests in the laboratory and (semi-)field tests with algae or macrophytes can address different types of population recovery, i.e. recovery of growth rate (potential recovery) and recovery of abundance or biomass (actual recovery). However, regulatory decision criteria for using such data in the risk assessment of plant protection products are not very clear yet. Modelling can help to extrapolate effects and recovery to different exposure scenarios and species but long-term data sets for testing such models are still lacking and also here, more specific decision criteria for how to use the results have to be defined.

### 5. References
