**Aquatic fungi in pesticide risk assessment**

Environmental regulations exist that aim at assessing adverse environmental effects of pesticides to determine if they are likely to pose an environmental risk and if so, deny or restrict their use. Since decades pesticides hence belong to the most strongly regulated chemicals. However, such prospective risk assessment is not bullet proof – it can only detect those adverse effects that are experimentally quantified, usually for a small number of species. The most recent example from the last decade showing this dilemma seems to be the indirect effect of a decline of species richness in aquatic insects on bird populations which is indicated by the study of Hallmann et al. (2014). Hence, risk assessment guidelines should be reviewed regularly based on current knowledge gained in ecotoxicology and environmental monitoring.

**Current approaches under European legislation**

For detecting the effects of pesticides and other substances on aquatic ecosystems, three regulatory approaches are currently in use: The biocidal products regulation (EU 1998), the plant protection products (PPP) regulation (EU 2009) and the Water Framework Directive (WFD) (EU, 2000). The biocides and PPP regulations are prospective, i.e. safe use has to be demonstrated before an active substance can be authoriseed for use in the EU. The WFD assesses risk retrospectively, i.e. it compares monitoring data with safe concentrations of individual substances, so called environmental quality standards (EQS), to assess the chemical status (i.e. the water quality with respect to the presence of chemical substances) of EU water bodies.

The most basic approach for the aquatic effect assessment is the assessment factor (AF) method. The ecological concept behind this approach is a simple food chain from algae (primary producers) via invertebrates (primary consumers) to fish (secondary consumers). Based on acute and chronic toxicity tests with representatives of the three trophic levels L/EC50 values (for acute effects) as well as no observed effect concentrations (NOECs, or EC10 for chronic effects) are derived. Depending on data availability, a higher or lower AF is applied on the lowest L/EC50 or NOEC/EC10 to determine a predicted no effect concentration (PNEC) for the ecosystem (European Commission 2003). For prospective risk assessment in the EU, the AF method is only used in the authorisation of biocides (EU 1998). For PPP authorisation, there is a slightly different approach not resulting in PNECs, but in a ratio between the observed EC50 or NOEC values and the predicted environmental concentration (Toxicity Exposure Ratio, TER). The given value for the TER that must be maintained can also be used as an AF to derive a regulatory acceptable concentration (RAC), that can be used in a similar way as the PNEC (EFSA 2013). Both prospective regulations have minimum data requirements that do not cover fungi. The AF approach to derive EQS for a retrospective risk assessment used by WFD (European Commission 2011) is more comprehensive. Bacteria and protozoa are explicitly mentioned as organism groups to be considered, though fungi are not. Nevertheless, if reliable data for fungi are available (e.g. in the open literature) they may be used. Moreover, for deriving a MAC-EQS for fungicides, the availability of fungi EC50 values seems to be crucial for lowering the standard AF from 100 to 10 (European Commission 2011), since it says: “For substances with a specific mode of action the most sensitive taxa can be predicted with confidence. Where representatives of the most sensitive taxa are present in the acute dataset, an AF < 100 may again be justified”. Especially for fungicides without an apparent sensitive taxonomic group this consideration seems important.

The second approach for aquatic effect assessment is the species sensitivity distribution (SSD) method. The ecological concept behind this approach is that protecting the species structure protects the whole ecosystem. This approach is more data intensive and is hence not as often applied as the AF approach. For deriving PNECs (biocides directive) and EQS (WFD) data for, at least 10 different species from 8 defined taxonomic groups have to be available to construct an SSD (European Commission 2003, 2011). If the data are log-normally distributed, a concentration can be derived at which not more than 5% of the species are potentially affected by the respective substance. This concentration is called the hazardous concentration for 5% of the species (HC5). To derive a PNEC or an EQS, the HC5 is finally divided by an AF depending on the quality of the SSD with regard to uncertainty around the HC5 as well as the number of taxa and input data. Although not explicitly mentioned as a taxonomic group required for generating an SSD, available data on fungi may be used to fill the following taxa requirement: “A family in any order of insect or any phylum not already represented”. If the data are statistically not normally distributed because some taxa are more sensitive than others, a separate SSD must be constructed for the more sensitive taxa. This is different under the PPP regulation (EFSA 2013, EU 2009), where an SSD is usually constructed for specific taxonomic groups (primary producers, invertebrates or fish) (Brock et al. 2006). Data on fungi – if available - could only be considered if neither of the specific taxonomic groups is specifically sensitive to the fungicide, because in this case, the SSD has to include data from all taxonomic groups.

Where micro- or mesocosm data is available a third method can be used for aquatic effects assessment. Studies in such small-scale, model ecosystems are often performed for PPP authorisaation (EU 2009). Species should be selected based on their known sensitivity to the tested substance but should also resemble communities representative for ponds, ditches or streams neighboring agricultural fields (EFSA 2013). Here, fungi are to be included in studies on substances, which are highly toxic to fungi, e.g. triazole and other fungicides. However, the protection goal of the PPP authorisation for aquatic fungi is not at the population but at the “functional group” level, since it is recommended to study the indirect effects of an impaired fungal community on macroinvertebrate shredders and microbial activity, e.g. using leaf litter decomposition as an endpoint for ecological function (EFSA 2013).

**Current approaches under European legislation**

Although aquatic fungal communities are not explicitly included in current EU regulatory approaches for pesticides, the occurrence of adverse effects on this organism group is not compatible with the protection goals of the existing regulatory frameworks. The principal protection goals of the EU PPP regulation (EU 2009) include “…to inhibit unacceptable effects on the environment referring to the impact on non-target species, including the ongoing behavior of those species and its impact on biodiversity and the ecosystem.” This implies that aquatic fungi are to be protected at the population and community level, however, the aquatic guidance document (EFSA 2013), calls for protection at the level of ecological function rather than fungal biodiversity.

For authorisation of biocidal products, it is required that the product “has no unacceptable effect itself, or as a result of its residues, on the environment having particular regard to the following considerations: (…) and its impact on non-target organisms [and] (…) its impact on biodiversity and the ecosystem”. A specific level of protection (e.g. functional level, population level, community level) is not defined, but it can be derived that both community structure (biodiversity and abundance) as well as ecosystem functioning should be protected, including fungi and their ecosystem functions.

The goal of the WFD (EU 2000) is to maintain or improve the current status of water bodies and prohibits their further deterioration. Protection of fungal communities and their function is thus included in the WFD protection goals. However, fungi are not part of the “quality elements” based on which the WFD defines the good ecological status (e.g. quality of the structure and functioning of aquatic ecosystems). These are: *i*) composition and abundance of plants, *ii*) diversity and abundance of invertebrate taxa, and *iii*) abundance of disturbance-sensitive fish species.

Since 2016, the protection of microorganisms is implemented in the Swiss water protection ordinance (Switzerland 2016) defines in its general requirements for the surface water quality that substances entering the water body as a result of human activity may not impair the reproduction, development and health of sensitive plants, animals and microorganisms. These general requirements are intended to form the basis for the setting of water quality criteria under this ordinance.

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