CHAPTER 6

Impacts of Pesticides on Freshwater Ecosystems

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Abstract: Pesticides can enter surface waters via different routes, among which runoff driven by precipitation or irrigation is the most important in terms of peak concentrations. The exposure can cause direct effects on all levels of biological organisation, while the toxicant mode of action largely determines which group of organisms (primary producers, microorganisms, invertebrates or fish) is affected. Due to the interconnectedness of freshwater communities, direct effects can entail several indirect effects that are categorised and discussed. The duration of effects depends on the recovery potential of the affected organisms, which is determined by several key factors. Long-term effects of pesticides have been shown to occur in the field. However, the extent of the effects is currently uncertain, mainly because of a lack of large-scale data on pesticide peak concentrations. In the final section, we elucidate the different approaches to predict effects of pesticides on freshwater ecosystems. Various techniques and approaches from the individual level to the ecosystem level are available. When used complementary they allow for a relatively accurate prediction of effects on a broad scale, though the predictive strength is rather limited when it comes to the local scale. Further advances in the risk assessment of pesticides require the incorporation and extension of ecological knowledge.

INTRODUCTION

Modern agricultural practices rely on the usage of synthetic pesticides (mainly herbicides, fungicides and insecticides) in order to prevent losses by pests [1]. The global pesticide production reached significant levels after the Second World War and rose sharply from approximately 500,000 t/a in the 1950s to over 3 million t/a at the beginning of the 21st century [2]. This trend will probably continue over the next decades because of a demand for higher food production as the human population increases, monocultural production for biofuels and potentially introduction of new pests in many areas associated with climate change [2, 3], though introduction of pest-resistant plants and an increase in organic farming and integrated pest management may counter this trend. Given the large amounts of pesticides applied globally and given the fact that they are designed to harm biota, there is a high potential for adverse environmental effects also on non-target communities [4]. When pesticides enter freshwater ecosystems, they do interact with the biotic and abiotic components of the ecosystem. Abiotic factors can lead to degradation (photo-decomposition by sunlight or hydrolysis by water) or adsorption of the compounds on sediment or organic matter. The interaction with the biotic parts comprises uptake, metabolisation and accumulation in organisms, which in turn may lead to adverse affects on the freshwater biotic community. These adverse effects are the topic of this chapter and will be delineated in depth after a brief overview of the entry routes of pesticides in freshwater ecosystems.

Once released into the environment, pesticides can be subject to airborne and waterborne entry in aquatic ecosystems. Airborne processes encompass wind drift during pesticide spraying (spray drift) and volatilisation after application with subsequent atmospheric transport that may lead to the deposition of compounds in remote ecosystems (thousands of kilometres) from their initial application. For example, organochlorine insecticides such as dichloro-diphenyl-trichloroethane (DDT) and lindane (□-HCH), which exhibited high usage patterns from the 1950s to the 1970s have become ubiquitous in the environment due to their high environmental persistence and potential for long-range atmospheric transport [5, 6]. Organochlorines are at present even detected in the polar regions, although they were never applied there [6]. However, for the majority of currently used pesticides atmospheric transport is confined to regional translocations within a 300 km radius as they are less persistent and have a lower potential for long-range transport [7, 8]. The waterborne translocation of compounds is driven by precipitation events or irrigation. Precipitation and irrigation can wash compounds from the field into adjacent surface waters via runoff or subsurface flow or into the groundwater.

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The quantitative relevance of the exposure route (airborne or waterborne) varies depending on physicochemical properties of the compound as well as the geographical, geological, hydrological and climatic conditions and crop type. For Germany, a modelling study on the exposure routes estimated the input of 65%, 10% and 25% of diffuse pesticide load by field runoff, flow in drainage channels and spray drift, respectively, though this study did not include atmospheric transport [9]. Spray drift is greatest when the spraying is conducted aerially using aircraft and for crops such as vineyards or orchards where the spraying occurs in a horizontal direction [10]. However, several studies emphasised the relevance of the waterborne exposure route concerning pesticide concentrations as reviewed in [11]. A study in North Germany showed that concentrations in a small headwater stream were elevated by several orders of magnitude during heavy rainfall events in the pesticide spraying period [12]. For cotton in Australia, the endosulfan concentrations in a creek and a river were approximately 10-fold higher during runoff events than as a consequence of spray drift [13]. Similar observations were made in the Lourens River in South Africa with runoff-associated pesticide concentrations at the first strong rain event after pesticide application being approximately 50-fold higher for two pesticides compared to the spray drift concentrations [14]. In irrigation farming of rice in Japan, short-term peak concentrations of pesticides in adjacent water bodies occurred in association with heavy rain or irrigation events [15, 16]. Overall, for water bodies in agricultural areas, intensive rainfall (> 10 mm per day) or irrigation with consequent runoff and subsurface flows after pesticide applications is recognised as the most important route of entry, both resulting in episodic short-term peak pesticide concentrations. In rivers that are fed by agricultural tributaries, the exposure may be more continuous due to dilution and overlapping input from different tributaries, but is still seasonal.

The pattern of episodic peak concentrations has to be considered in investigations on the effects of pesticides on aquatic biota. Although a large number of studies has been conducted on the effects of pesticides in surface waters, the majority failed to clearly link effects to exposure, partly because the study design did not include a sufficient quantification of pesticide peak concentrations [11]. Hence, an exposure monitoring method should be adopted that captures runoff events in the spraying period. Automatic continuous water sampling or automatic event-triggered water sampling can be employed, but these methods are cost- and labour-intensive [12, 17, 18]. Therefore, alternative methods have been suggested such as a less costly version of an event-triggered water sampler [19], a suspended matter sampler for particle-associated hydrophobic pesticides [20, 21] or passive sampling using adsorbent membranes if continuous background exposure is absent or negligible [22]. To sum up, the determination of pesticide peak concentrations in the water bodies is crucial in studies on the effects of pesticides on freshwater organisms.

DIRECT EFFECTS OF PESTICIDES

The freshwater community consists of different groups of organisms such as fish, amphibians, invertebrates, plants or microorganisms. Pesticides can have direct and indirect effects on these organisms. Direct effects are caused by the physiological action of a pesticide within an organism. However, the biotic community is characterised by ecological interactions between species such as competition or predation and indirect effects refer to effects mediated via these interactions [23]. For example, mortality of water fleas as a direct result of exposure to a pesticide (direct effect) may lead to an increase of algae biomass due to a release from grazing pressure (indirect effect). We will firstly focus on direct effects as they are a prerequisite for understanding the indirect effects.

In general, direct effects of chemicals on an organism depend on the concentration; i.e. the dose determines the poison (Paracelsus). However, some further general factors influence the occurrence and magnitude of adverse effects:

- exposed life-stage: different life stages of organisms can be affected differently by the same exposure with younger life stages of fish, amphibians and invertebrates being in general more susceptible [24, 25];
- exposure duration: generally a longer exposure time leads to stronger effects [26, 27];
- biomagnification can imply a temporal delay in effects: for example, organochlorine pesticides can biomagnify along the food web, resulting in several orders of magnitude higher concentrations per lipid weight in organisms at the top of the food web [28];
- presence of additional stressors: the effect of a single compound can be enhanced in the presence of other pesticides [29, 30] or other stressors such as UV radiation [31], parasitism [32], predation [33] and food scarcity [34];

- population density: toxicant-induced effects on individuals in high density populations can reduce the negative intraspecific interaction and therefore be compensated [35], though the population structure may remain altered as the toxicant exhibits age-dependent mortality or delay in development [36, 37];
- history of the community: previous exposures to toxicants can modify the response of a community either by an acquired tolerance (pollution-induced community tolerance (PICT)) or a higher sensitivity [38-41].

These factors can, together with the biological and physicochemical characteristics of the exposed ecosystem, modify the potential direct effect of a pesticide entering a freshwater ecosystem. Therefore, the effects may differ between ecosystems with different modalities of these factors. For example, a certain concentration of a pesticide will most likely have stronger effects on a community of a forest stream that never received pesticide input in comparison to an agricultural stream that is subject to recurring pesticide exposure [42]. However, our knowledge about the comparative relevance and the differences in the modalities of these factors between natural ecosystems is very limited and does not allow for a generalisation of their influence on effects. Nevertheless, when studies are conducted within similar ecosystems (e.g. agricultural streams) or the factors vary only slightly between the sites, the pesticide concentration (or the derived toxicity) should be the most important predictor of the pesticide effect. Indeed, in field studies on the effects of pesticides on invertebrate communities in agricultural streams, most of the variability in community endpoints could be explained by pesticide concentration (or the derived toxicity) alone even across biogeographical regions [43-45]. The period and duration of exposure as well as the history of the communities were relatively similar in these studies. Nevertheless, the amplification of the effects of pesticides through these factors is one explanation why field effects have been reported at levels lower than expected based on laboratory or artificial stream experiments [42, 44]. Moreover, these factors are presumably most important when results from laboratory toxicity tests are extrapolated to the real world.

Pesticides can act on different endpoints that can be classified according to the level of organisation in biotic communities:

- suborganismal; e.g. changes in enzyme activity or chemical signaling [46], reduced respiration [47];
- organismic; e.g. increased mortality [48], change in morphology [49], delayed development [50], change in behaviour [51], increased susceptibility to infections [52] or reduced reproduction [53];
- population; e.g. changes in age structure [37], population growth rate [54] or mortality rate [55];
- community; e.g. changes in community composition [43, 56-59];
- ecosystem; e.g. changes in ecosystem processes [44, 60].

These levels are linked mechanistically in a hierarchical bottom-up order that can be described as a pyramid effect (Fig. **1**) [61, 62], and we outline this conceptual model as follows. A toxicant always acts on the physiological level of an organism first. Subsequently this may lead to individual-level effects such as delayed development or mortality. If the effect is strong and several individuals are impacted, the effect can propagate to the population level (e.g. the population growth rate may change). In cases of severe contamination, some or many populations or even whole groups of organisms (e.g. crustaceans) may go extinct and the niches may be occupied by other species. Thus, a change in community composition would be observed. For example, freshwater sediment microbial communities were exposed to a combination of fungicides, insecticides and herbicides, which lead to an alteration in bacterial community composition [59]. Finally, whether the specific functions of the affected species can be compensated for by other species (*i.e.* the degree of functional redundancy of the ecosystem [63]) ultimately determines the occurrence of an effect on an ecosystem process (e.g. breakdown of organic matter, primary production, nutrient recycling). In general, effects on all subjacent levels are a prerequisite for effects on higher levels of the pyramid (Fig. **1**). However, effects on ecosystem processes may occur without visible effects on the population or community level. For example, some studies in artificial ecosystems demonstrated that although the effects of herbicides on the rate of photosynthesis could be measured, no population effects could be detected among populations of primary producers [64, 65]. In most studies with artificial ecosystems, however, effects on ecosystem processes resulted from community and population level changes [66].

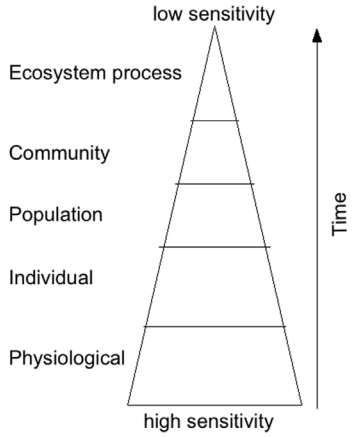

Figure 1: Effect pyramid. Effects on an upper level of biological organisation of the pyramid may occur with a lag phase and require effects on the subjacent levels. This is because higher levels of biological organisation have a lower sensitivity. See text for examples.

Conceptually, effects on lower levels of the pyramid (Fig. **1**) generally occur at lower concentrations than effects on higher levels since higher levels integrate several individuals or populations, of which some will be more tolerant. Thus, higher levels of biological organisation have a higher tolerance for pollution. Moreover, the effects on the different levels of organisation are not necessarily temporally synchronised as it may take from some hours to days for a suborganismic effect to affect individuals and from days to weeks for individual effects to affect populations, communities and ecosystems [61]. Studies on the effects of the pyrethroid fenvalerate demonstrated that population and community disturbance lagged behind and persisted while physiological or individual effects were not detectable anymore [36, 67].

Direct effects of pesticides have been reported on almost all biological endpoints for all groups of freshwater organisms. However, most studies were conducted in the laboratory whereas the focus of this book lies on effects in ecosystems [68]. Therefore, we mainly consider effects that have been observed under field or field-relevant conditions; *i.e.* either detected in field studies or in artificial ecosystems. In addition, we only include direct effects that were reported in the last two decades, so that the findings represent effects of currently used pesticides. Nevertheless, we briefly portray the effects on the aquatic wildlife that were reported since the 1950s and stimulated the writing of the historical book "Silent Spring" by Rachel Carson [69].

The significant agricultural use of organic pesticides, mainly organochlorine insecticides, started after the Second World War [70]. DDT, in particular, was used globally in large amounts for mosquito control and agricultural pest control (70-80% of total DDT used), reaching an annual use of approximately 400,000 t in the 1960s [71]. Reports of effects on wildlife and humans occurred soon after the widespread introduction of organochlorine insecticides [70, 72]. In the aquatic ecosystems, runoff of organochlorine insecticides following rain events in adjacent streams lead to severe fish kills and the eradication of the stream invertebrate fauna over stretches of several kilometres [70, 73]. Also aquatic and terrestrial birds in sprayed regions succumbed to lethal doses [72, 74]. More surprisingly, effects on the reproduction of several fish-eating birds were observed that comprised thinning of the egg shells resulting in the eggs being crushed during nesting, abandonment of nest and egg-eating by the parents [5]. The failure to reproduce affected bird colonies, resulting in population declines in species such as the brown pelican (*Pelicanus occidentalis*), great blue heron (*Ardea herodias*) and herring gull (*Larus argentatus*) [5]. Reproductive effects were even observed in regions with relatively low environmental exposure to DDT and occurred as a consequence of biomagnification of this highly persistent compound along the food chain, so that fish-eating birds at the top of the aquatic food web were finally exposed to biologically effective concentrations [5]. For example, after spraying of the Clear Lake in California for gnat-control, local populations of the western grebe (*Aechmophorus occidentalis*) declined, exhibiting fat concentrations of up to 100,000-fold the lake water concentrations, 400-fold the plankton concentrations and 200-fold the small fish concentrations in fat [75].

In the 1970s several persistent compounds (e.g. DDT, endrin, dieldrin) were banned in most countries due to their unacceptable effects on wildlife [76]. In agriculture, organochlorine insecticides were mainly substituted by the less persistent chemical families of pyrethroid and organophosphate insecticides. Although generally less widespread, effects

on non-target organisms were frequently reported in the 1980s. Between 1977 and 1984 approximately 56% of 128 fish kills in the United States were attributed to pesticide pollution, primarily to the organochlorine endosulfan and the organophosphate malathion [77]. Historically a trend can be observed from compounds with a broad mode of action affecting many non-target species to compounds with a specific mode of action that are less toxic for the majority of non-target organisms. Currently used insecticides such as pyrethroids are characterised by a 1000-fold lower toxicity for mammals compared to organochlorine pesticides [78, 79]. For example, an accidental input of the pyrethroid cypermethrin lead to the complete eradication of invertebrates over a stretch of 3 km but no fish kill was observed [80]. Nevertheless, given that there are usually some organisms in the freshwater community that are physiologically related to terrestrial pest species (e.g. insects), present and future pesticide use is likely to continue posing a threat to aquatic ecosystems. The effects of pesticides used in the last two decades on the different groups of organisms are summarised in Table **1**. Effects were reported for most groups and endpoints, though there are some notable differences. Field studies that show effects on macrophytes, phytoplankton and benthic algae as well as other microorganisms are scarce and are almost entirely limited to artificial ecosystem experiments. By contrast, several field studies have demonstrated effects in freshwater ecosystems on macroinvertebrates and zooplankton [45, 68, 81-85], fish [86] and amphibians [52, 87-90]. Here, the frequency of reported effects on macroinvertebrate and zooplankton assemblages is much higher than for fish and amphibians, for which only a few field studies reported effects at the population or community level. In the case of amphibians this is not surprising as they mainly appear in lentic (standing) surface waters, which generally receive less pesticide input than lotic (running water) habitats [91].

Table 1: Effects of pesticides on the different groups of organisms under field or field-relevant conditions reported in the last two decades: frequency of reported effects, field relevance and examples.

Effects level[a]	**Bacteria, protozoa and fungi**	**Phytoplankton and benthic algae**	**Macrophytes**	**Macroinvertebrates and zooplankton**	**Fish**	**Amphibians**
Suborganismal (S)	-	Genetic changes [222]	Increase Glutathione-S-transferase and chlorophyll ratio [223]	p-nitrophenylacetate esterase, Glutathione-S-transferase and Acetylcholin esterase inhibition [167]	Acetylcholine esterase inhibition [46]	Alteration of receptor binding and cell signalling [224, 225]
Individual (I)	Decrease in bacterial activity[226]	Decline in photosynthesis and mortality [227, 228]	Decline in frond area and weight, and mortality [229, 230]	Feeding depression and mortality [231]	Mortality [232]	Increase in parasite susceptibility and mortality [52, 233]
Population decline (P)	[234]	[235]	[236]	[11, 210]	[86]	[87]
Community: change in composition (C)	[59, 98]	[235, 237]	[238]	[42]	[86]	-
Associated Ecosystem processes (E)	Inhibition of microbial mineralisation [239]	Reduction in pH and O_2 [64]	Decrease in nutrient level, pH and carbonate cycle [238, 240]	Inhibition of organic matter decomposition and decrease of energy transfer [44, 241]	-	-
Frequency of reported effects[a]	I, P, C, E: low	S: low I,P,C,E: medium	E, C, S: Low P, I: medium	High for all levels, except E: low	S: high I: medium P,C:Low	P: medium S, I: high
Clear evidence from field studies	No field studies, only mesocosm (except one field study on C)	No field studies, only mesocosm	Not for E, C, S level	All levels	All levels	For S, P, I

[a] See first column for abbreviations: none: no studies; low: 1-5 studies; medium: 5 to 10 studies, high: > 10 studies

The dominance of reports on effects on macroinvertebrates and zooplankton followed by fish and amphibians compared to macrophytes, phytoplankton and benthic algae raises the question whether this is: a) an artifact of organism selection in biomonitoring; or b) observed impacts represent the real frequency of effects in these organism groups. There are several reasons that suggest an over-representation of effects on animals and specifically on macroinvertebrates and zooplankton:

- macroinvertebrates were much more frequently selected as biomonitoring organisms than other groups due to their well-described taxonomy, relatively high species richness, their sedentary nature and low expense of monitoring programs [92, 93];
- similarly, larger organisms such as fish and amphibians have received much more attention than plants and microorganisms because of their economic importance (fish) and due to individual preferences of researchers for vertebrates [94-96];
- the detection of effects on microorganisms was much more difficult because of the variability between sites and even adjacent micro-sites [97], and only recent advances in molecular techniques allow for a reliable detection of community changes in microbial assemblages in the field [98];
- macrophytes, phytoplankton and benthic algae as well as other microorganisms are in general more susceptible to herbicides than the other groups of organisms [65, 99] and since herbicides account for a major part of the applied pesticide mass [100], it is very likely that effects in the field have occurred but were not noticed;
- algae and microorganisms are known to have a fast recovery, hence effects may only be transient and not detectable after a few weeks [65].

We therefore conclude that the present picture concerning effects of pesticides on ecosystems is likely biased towards the fauna and especially towards invertebrates. In the next section we examine to what extent the biotic community is affected by different classes of pesticides.

Compound-Specific Effects on Different Groups of Organisms

The early days of ecotoxicology were driven by the myth of a "most sensitive species" that could be used as a standard test organism to predict the impacts of toxicants in ecosystems; *i.e.* no effects should occur in the ecosystem as long as no direct effects occur in the most sensitive species [101]. This quest for the "most sensitive species" relied on several assumptions, one being that the most sensitive test species for a set of compounds would be most sensitive to other compounds as well. This assumption has received extensive criticism and we will give a brief overview of studies with pesticides that contradict this assumption. Concerning herbicides, a study of van den Brink *et al.* [99] showed that the sensitivity of test species varies with the chemical class of the compound and that there is no single most sensitive primary producer. For example, the common duckweed *Lemna minor* was more sensitive to the herbicides diquat and linuron but less sensitive to diuron and metamitron than the green algae *Selenastrum capricornutum* [99]. Similarly, crustaceans are among the most sensitive species to organochlorine and pyrethroid insecticides, whereas the recently introduced neonicotinoid insecticides exhibit orders of magnitude higher toxicity to insects than to crustaceans [102]. In addition, the sensitivity of a species to a toxicant depends on the life stage and a sensitivity ranking of several species may therefore in some cases vary with the selected life stage [24].

However, this does not mean that patterns of sensitivity are completely stochastic, a broad classification into sensitive and tolerant taxa is possible when compounds are grouped according to their mode of action [103, 104]. In the case of pesticides, some substances have specific sites of action; e.g. photosynthesis or ergosterol synthesis that are only present in certain groups of organisms [105] (see Chapter 1). For example, pesticides that target the hormonal reproductive system of insects are unlikely to affect aquatic primary producers, which have a completely different reproduction system [106]. Other pesticides have a wider activity and target processes that are present in all or many organisms (e.g. cytochrome oxidase [105]), which complicates the prediction of effects.

There are some general rules of thumb on the sensitivity of the groups of aquatic organisms to herbicides, insecticides and fungicides. Herbicides are mainly targeting organisms that perform photosynthesis. A meta-study of artificial ecosystem studies on herbicides showed that for this class of compounds, primary producers are more

sensitive than aquatic animals [65]. Which group of primary producers (*i.e.* macrophytes, algae or microorganisms) is most sensitive depends on the mode of action of the herbicide [99].

A meta-study on insecticides showed that for this class of compounds, macroinvertebrates, zooplankton and fish are the most sensitive groups compared to other organisms [66]. Given that several currently used insecticides are designed to target invertebrates [1], macroinvertebrates and zooplankton are presumably more susceptible than fish. In fact, 16 currently used insecticides for which comparative toxicity data was available, exhibited highest toxicity to invertebrates and zooplankton [107]. In addition, several artificial ecosystem studies support this hypothesis since fish were less sensitive than invertebrates [108].

Fungicides are less extensively studied than herbicides and insecticides. Therefore sound generalisations about which groups are most sensitive to these compounds are problematic. Nevertheless, based on the mode of action, several fungicides should be most toxic to aquatic microorganisms, especially aquatic fungi [109]. An unpublished evaluation of experiments in artificial ecosystems did not indicate elevated toxicity by fungicides to the aquatic fauna and primary producers [109].

Recent studies have began to incorporate these differences in the modes of actions and proposed a new strategy for the search of sensitive species. A study of Wogram & Liess [103], extended and confirmed by von der Ohe & Liess [104], found that for macroinvertebrate species the variability of the sensitivity to organic chemicals is higher between taxonomic groups (primarily families and orders) than within groups and could be pooled in a relative toxicity ranking. For example, stoneflies were among the most sensitive taxa for organic chemicals, whereas gastropods are relatively tolerant to organic chemicals [104]. This study, however, did not distinguish toxicant mode of actions between organic chemicals and some studies demonstrated that such differences exist. Rubach *et al.* [110] showed that the relative sensitivity of macroinvertebrate taxa differed, albeit minor, between organophosphates, pyrethroids and carbamates. In addition, imidazole fungicides were more toxic to gastropods than many insect taxa [111, 112]. Furthermore, the differences in sensitivity ranking were even stronger for heavy metals and salinity [104, 113]. No relative sensitivity rankings have been established for other groups of organisms so far. Overall, we propose that with regard to toxicants with a similar mode of action a consistent, relative sensitivity hierarchy may be established at least for the different groups of organisms that can be used for risk assessment of pesticides, though no universal most sensitive species or group of species exists.

INDIRECT EFFECTS IN THE AQUATIC COMMUNITY

In ecosystems, species interact with other species and their abiotic environment. Direct effects of pesticides on a species can alter these interactions and therefore have an indirect (also termed secondary) effect on species that are otherwise not directly affected. The following ecological relationships may lead to indirect effects via propagation of direct effects:

- predation: comprises herbivore-plant, predator-prey and parasite-host relationships
- competition: inter- and intra-specific competition
- species-habitat: influence on habitat characteristics by some species
- mutualism or commensalisms

Indirect effects of pesticides have been reported frequently and have been summarised in different publications [65, 66, 114-116]. Here, we will give a brief overview on potential indirect effects of herbicides and insecticides. Fig. (**2)** sketches the direct and indirect effects of a herbicide and an insecticide in a freshwater ecosystem. As outlined in the last section, primary producers are generally at highest risk of being adversely impacted by herbicides. A reduction of the primary producers can lead to a decrease in the herbivore populations due to food limitation and/or habitat loss (Fig. **2**). For example, in a study on the effects of the herbicide atrazine on freshwater communities in artificial ponds, growth and reproduction of zooplankton (e.g. *Simocephalus serrulatus, Daphnia pulex*) decreased as a consequence of phytoplankton biomass reduction [117]. Similarly, amphibian tadpole biomass (e.g. *Rana catesbeiana*) decreased due to a reduction of food source (periphyton) and loss of macrophyte habitat (e.g. *Typha*

latifolia, Chara sp.) [116]. The indirect effect on herbivores as a consequence of a reduction in food may be less pronounced in nutrient-rich ecosystems in the field. Where a reduction in herbivores occurs, effects may subsequently propagate to higher trophic levels; e.g. predators that prey on the herbivores (Fig. **2**). For example, herbicide-induced reductions in zooplankton and macroinvertebrates (e.g. Chironomidae spp.) due to loss of primary producers as food and habitat resulted in a decreased total biomass of bluegill sunfish (*Lepomis macrochirus*) [116]. This ecological effect chain represents bottom-up indirect effects because the lowest trophic level (primary producers) determines the effects on higher trophic levels. While these indirect effects are due to predatory ecological relationships, competitive relationships between primary producers promote an increase of tolerant primary producers when sensitive competitors are eliminated by a pesticide (Fig. **2**). For example, algal blooms of *Chlamydomonas* sp. were observed after linuron strongly reduced macrophyte populations of *Elodea nuttallii* [118].

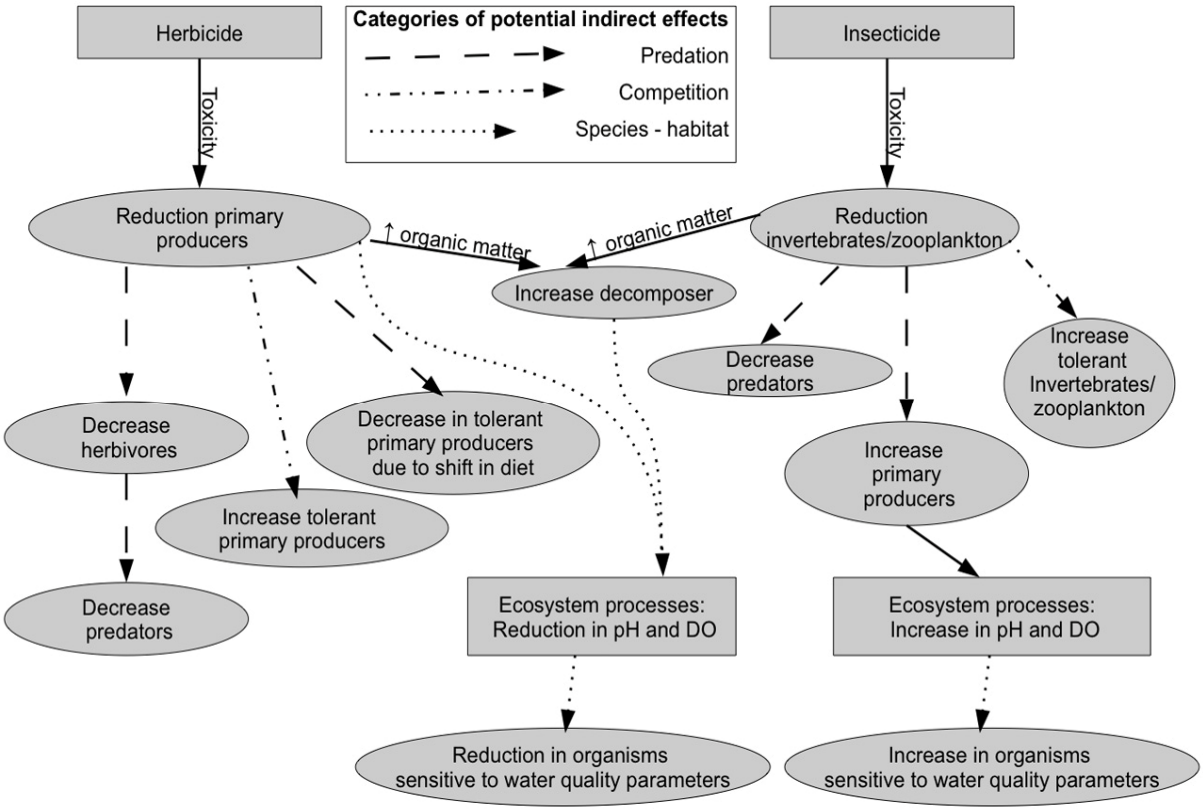

Figure 2: Schematic representation of direct (solid line) and indirect (dashed and dotted lines) potential effects of pesticides in freshwater ecosystems. See text for further explanation.

Photosynthesis is an important ecosystem process that influences water quality, and the inhibition of photosynthesis by herbicides results in a lower concentration of dissolved oxygen (DO) and lower pH values during daytime (Fig. **2**). In an indoor mesocosm study, the highest linuron treatments of 50 and 150 µg/L reduced DO and pH by up to 40% and 25%, respectively [119]. If a herbicide causes acute mortality of macrophytes, decomposition by decomposers may further enhance the reduction in pH and DO concentration [65]. This deterioration of water quality can then have detrimental impacts on sensitive invertebrate species and this represents a case of indirect effects resulting from species-habitat relationships (Fig. **2**). For example, a strong reduction of cladoceran and copepodan populations was partially attributed to a reduction in DO to only 20% compared to controls, following a 10 mg/L contamination with hexazinone in lake enclosures [120].

The schematic effects of the insecticide in Fig. (**2)** illustrates a combination of top-down and bottom-up indirect effect [121]. We present a scenario, demonstrated by many studies [66], where an insecticide adversely affected invertebrates and zooplankton species. The bottom-up indirect effect is represented by the decrease of fish population density due to

a reduction of invertebrate prey. For example, a significant reduction in macroinvertebrates such as ephemeropterans (mayflies) and dipterans as well as two zooplankton groups (Daphniidae and Cyclopidae) in outdoor ponds after treatment with methyl parathion led to decreased mean weights in rainbow trout (*Salmo gairdneri*) [122]. The top-down indirect effect commences with a release of primary producers from grazing pressure and may result in growth of their populations. In a mesocosm study on the effects of chlorpyrifos, the eradication (Insecta and Amphipoda) and reduction (Isopoda, Cladocera and Copepoda) of parts of the invertebrate community by the pesticide resulted in a two- to three-fold increase in periphyton chlorophyll-*a* accompanied by a bloom of *Oscillatoria* sp. [123]. Some tolerant invertebrate and zooplankton species may profit from the reduced competition with directly affected sensitive invertebrate species (Fig. **2**). In the aforementioned study on chlorpyrifos, Sphaeriidae molluscs and herbivorous rotifers (*Polyartha* sp.) increased as a consequence of reduced competition for food with more sensitive invertebrates [123]. Similar observations were made in a field study where, after pesticide exposure, sensitive species decreased and tolerant species increased [43]. The effects on ecosystem processes are ambiguous in this scenario. While the increase of primary producers increases the pH value and DO concentration, the decomposition of dead invertebrates/zooplankton by fungi or bacteria decreases these water quality parameters [66]. We assume that in larger freshwater systems and lotic systems the first mechanism would be more important. In the case of a strong reduction of the invertebrate fauna another important ecosystem process, leaf-litter decomposition can be inhibited [124]. A field study in 16 French streams demonstrated a three- to five-fold decrease in leaf-litter decomposition in streams with an insecticide-impaired invertebrate community [44]. Leaf-litter decomposition represents an important energy source in stream ecosystems and a reduction can even adversely impact river sections several kilometers downstream, since they rely on particulate organic matter input from upstream sections [125, 126]. Hence, indirect effects can occur a long way from the location of the direct effect.

Pesticides represent only one of the many disturbances (e.g. floods, droughts, land-use change, acidification, dredging *etc.*) that shape freshwater ecosystems [127] and other disturbances can also result in indirect effects [114]. The similarity of indirect effects of different disturbances depends on the disturbance type and selectivity of their effects on biota [128]. While some disturbances such as floods also occur in pulses, they presumably act less selectively on the trophic levels or groups of organisms in the biotic community; e.g. they are unlikely to only affect primary producers or invertebrates. By contrast, many of the currently used pesticides are relatively selective *i.e.* they act on a specific trophic level or group of organisms as outlined above. However, similar indirect effects have been reported for other contaminants such as pulses of heavy metals or accidental discharges of organic toxicants in freshwater ecosystems [114].

Indirect effects of pesticides have mainly been studied in artificial ecosystems because under field conditions a clear differentiation between direct and indirect effects is more difficult. In the field, the time and magnitude of pesticide input driven by precipitation events is unknown and the input usually comprises a mixture of pesticides that may directly affect several groups in the biotic community concurrently. Furthermore, the variation regarding environmental parameters, pesticide exposure and biotic community composition is usually rather high between sampling sites. Hence, the detection of indirect effects would require an extended Before-After-Control-Impact (BACI) sampling design [129] consisting of a spatially and temporally highly replicated monitoring of the biotic community in control and impacted sites before the pesticide input and immediately after the contamination to determine the direct effects and then a few times in weekly intervals to identify indirect effects. However, the efforts of such a study would be jeopardised by ignorance of the impact of the monitored runoff events. In the worst case scenario, any of the selected sites would be impacted (compare [44] where no impacts were detected in a field study in Finland). Even if direct and indirect effects could be detected using multivariate statistical techniques [130-133], causality could not be inferred and additional studies under standardised conditions would be needed [134]. The field studies conducted to date on the effects of pesticides did not aim to differentiate between direct and indirect effects. This may explain the lower effect thresholds that have been observed in field studies compared to artificial ecosystems [43, 44, 135], because the effects on invertebrates may not have resulted from direct toxicity of pesticides alone but as well may be a bottom-up indirect effect from depletion of primary producers or heterotrophic microorganisms. An alternative approach for assessing the direct and indirect effects in the field integrates ecological modelling [136]. The modelling is used to predict direct effects of an exposure event and the differences in the effects that are observed in the field are considered as indirect effects. However, currently used models do not allow for an integration of all the factors that can influence the strength of direct effects (see section “Direct effects of pesticides”) and therefore gives rise to high uncertainty.

EFFECT, DURATION AND RECOVERY DYNAMICS

Given the widespread application of pesticides, it is almost inevitable that some fraction enters freshwater ecosystems. Consequently, the authorisation for use of pesticides involves the passing of a value judgement on the question: "Which ecological effects are unacceptable?" [137]. For regulators in the European Union, long-term field effects on populations and communities are deemed unacceptable, though the operationalisation of "long-term" may vary on a case-by-case basis [138]. Similarly, the US EPA includes the assessment of recovery from pesticide stress in their risk assessment framework and regards potential irreversibility (*i.e.* permanent changes in the community structure or ecosystem processes) as an adverse effect [139]. From these perspectives follows that transient short-term effects are considered acceptable. The underlying hypothesis is that a toxicant can have only transient effects on an ecosystem and that the ecosystem may subsequently recover to an initial or reference state; *i.e.* the community recovery principle [140]. This hypothesis has been subject to criticism. Landis *et al.* [39] argue that because of the dynamic nature of ecosystems, any pesticide-induced effects are irreversible, rejecting the concept of recovery. Even if recovery can be observed on one level of biological organisation, changes may persist on other levels; e.g. the gene pool can be impoverished [141]. For example, fish populations of the brown bullhead (*Ameiurus nebulosus*) in the Great Lakes have been observed to have different genetic structures in populations that have been exposed to a mixture of organic toxicants and metals [142]. The US EPA acknowledges this criticism by defining recovery as "the return of a population or community to some aspect(s) of its previous condition" [139]. We agree with the criticism of Landis *et al.* [39], but would rather integrate it in the evaluation of studies on recovery; e.g. by studying effects on suborganismal endpoints in affected populations. Hence, we advocate the use of studies on the recovery of an affected artificial ecosystem or field ecosystem as a useful tool to deliver information on the toxicity of a pesticide that can be used to evaluate the acceptability of effects.

So far, almost all studies on the recovery of an ecosystem from the effects of a pesticide were conducted in artificial ecosystems and, in general, community composition was selected as the endpoint. Complete recovery was assumed when significant differences between treated and non-treated communities were not detected anymore. While controlling the type I error rate (reject the null hypothesis that recovery occurred when it is true), this entails the risk of a type II error (fail to reject the null hypothesis that recovery occurred when it is false) that may be more interesting in studies on recovery. Unfortunately, most studies on artificial ecosystems lack an analysis of the probability of a type II error of the selected test, which may be relatively high given that sample sizes in these studies are usually low (< 5 replicates per treatment). Therefore, the time to complete recovery is probably underestimated.

A meta-analysis of artificial ecosystem studies with various insecticides highlighted that the initial acute toxic effect of the substance is a critical factor for the time to recovery [143]. Methodologically, the toxicity of different compounds can be compared using the Toxic Unit (TU) approach [144], in which the results of laboratory toxicity experiments for a specific test organism (usually LC50) are used as a benchmark to compare the toxicity of concentrations of different compounds. The TU is given by

$$TU = \frac{c_i}{LC50_{i,j}}$$

where c is the concentration of compound i and j the benchmark organism. The benchmark organism should be selected according to its sensitivity for the study compounds. In the studies considered here, *Daphnia magna* or, in very few cases, a fish species (*Pimephales promelas, Oncorhynchus mykiss, Lepomis macrochirus*) was selected as standard test organism to compute the TU for insecticides. For herbicides, green algae (*Scenedesmus subspicatus, Selenastrum capricornutum, Chlorella vulgaris*) or macrophytes (*Lemna* spp.) were employed for TU calculation. For reasons of simplicity, throughout this chapter we use $TU_{Daphnia}$, TU_{fish} and $TU_{primprod}$ for the TUs based on *Daphnia magna*, fish and primary producers, respectively. Note that the TU approach assumes concentration addition *i.e.* the same concentration-response relationship for compounds, while this may differ in reality; e.g. one compound has no effects at a TU of 0.01 (1/100 of the LC50) while another compound may still have effects due to a flatter concentration-response curve [145].

In a meta-analysis of 26 artificial mesocosm studies with acetylcholinesterase-inhibiting insecticides and 18 studies with pyrethroid insecticides, no long-term community effects (> 8 weeks) were observed for a $TU_{Daphnia} < 1$ for fish,

microorganisms and primary producers [108]. Even with higher compound concentrations relating to a $TU_{Daphnia}$ between 1 and 100 only 4 of 15, 2 of 22 and 2 of 27 observations indicated long-term effects for fish, microorganisms and primary producers, respectively (observations with unknown recovery excluded). By contrast, freshwater insects, macrocrustaceans and microcrustaceans (Ostracoda, Cladocera and Copepoda) exhibited clear long-term effects above a $TU_{Daphnia}$ of 0.1 and even lower for pyrethroids, where concentrations between a $TU_{Daphnia}$ of 0.1 and 0.01 caused long-term effects in aquatic insects (1 of 10 observations) and macrocrustaceans (2 of 5 observations) [108].

A similar meta-analysis was performed for artificial ecosystem studies with photosynthesis-inhibiting herbicides, auxin-simulating herbicides and growth-inhibiting herbicides [65]. No long-term effects (> 8 weeks) were observed for molluscs over the whole range of tested concentrations (up to a $TU_{primprod}$ of 100). For zooplankton, long-term effects were reported in 3 of 16 cases with a $TU_{primprod} > 1$. Long-term effects on fish and amphibians occurred in 4 of 13 cases at concentrations relating to a $TU_{primprod} > 0.1$. For macrocrustaceans and insects no long-term effects were detected, except in 1 of 3 observations on auxin-inhibiting herbicides – but at a $TU_{primprod}$ of 0.01. All these effects were most likely indirect effects that resulted from the depletion of populations of primary producers or from the associated habitat degradation [65].

Phytoplankton and periphyton showed clear long-term effects for concentrations with a $TU_{primprod} > 1$, and in 1 of 8 cases with a $TU_{primprod}$ between 0.1 and 1, long-term effects were reported for phytoplankton. Macrophytes were more sensitive, with clear long-term effects in several studies above a $TU_{primprod}$ of 0.1, with 2 of 5 observations on auxin simulators indicating long-term effects between a $TU_{primprod}$ of 0.001 and 0.1.

To sum up, based on artificial ecosystem studies, long-term effects may occur when the concentrations exceed concentrations relating to a $TU_{primprod}$ and $TU_{Daphnia}$ of 0.01 for insecticides and herbicides. This seems to be in general agreement with two field studies on 20 streams in North Germany and 29 streams in Spain where the macroinvertebrate community exhibited long-term alteration at a $TU_{Daphnia}$ of similar magnitude [43, 135].

Only a few studies have scrutinised the duration of effects that are classified as long-term and they only focused on effects on macroinvertebrates. Recently, an artificial stream ecosystem study with the neonicotinoid insecticide thiacloprid reported the persistence of adverse effects on sensitive macroinvertebrate species half a year after a pulse exposure with a $TU_{Daphnia}$ of 0.014 [146]. An artificial pond ecosystem study demonstrated that at concentrations associated with a $TU_{Daphnia}$ of 20, the invertebrate communities of control ponds and treated ponds were still significantly different after 2 years whereas there was recovery at lower concentrations [67]. Very high concentrations of the insecticide methoxychlor ($TU_{Daphnia}$ of 10,000), which may occur from direct spraying of water bodies (e.g. mosquito control), implicated a different community in the treated stream compared to a reference stream over 5 years in a field study [84]. In the before mentioned study on 20 streams in North Germany, no full recovery of the community was observed within one year for a $TU_{Daphnia} > 0.001$ [43]. Relating these studies to generation times of invertebrates, which usually range from a few weeks to a year, illustrates that the recovery time is in the range of one to a few generations. Overall, these studies suggest that recovery in community endpoints can take over one year, and up to several years in cases of very high concentrations.

Factors Fostering Community Recovery Processes

The duration of community recovery from pesticide stress depends to some extent on the magnitude of the effect which in turn is determined by the concentration, exposure duration and toxicity of the pesticide and its transformation products [143, 147]. However, some other factors also influence the time to recovery:

- Ecological traits of species in the affected biotic community: In particular a short generation time, high reproduction rate, presence of resistant life stages and a high dispersal capacity of species augment recovery of populations [148]. For example, phytoplankton species generally recover faster from adverse effects of pesticides than macrophytes due to shorter generation times [65]. Similar observations were made for invertebrates with a short generation time [146, 149, 150] or high dispersal capacity [43, 151, 152]. Finally, microorganisms are presumably less vulnerable to pesticides due to short generation times and adaptability [41], though there may be exceptions; e.g. aquatic hyphomycetes [109].

- Timing and frequency of pesticide exposure: The timing of exposure is a crucial factor since the susceptibility of many species changes over the year because they may have terrestrial or resistant life-stages [43]. Concerning frequency of pesticide exposure, cyclical pulses of toxicant exposure can shape communities in the sense that they adapt [153] and recover until the next pulse occurs, while at the same time the exposure exerts a selection pressure on the communities [42]. Moreover, repeated pulses within a short period (weeks) are known to amplify effects in laboratory and mesocoms experiments [38, 108].
- Spatial dimension of effect: Large-scale contamination such as accidents require a longer time to recover since a higher magnitude of external recolonisation is needed to compensate for the effect [154]. Another aspect is that large-scale contamination often excludes the opportunity to avoid intoxication in refugia or by escaping, which again enhances subsequent recovery [155, 156].
- Position in the hydrological network: Several studies have demonstrated that the presence of undisturbed upstream sections foster recovery of the affected stream sections [43, 44, 157], presumably via recolonisation [158, 159] or energy provisioning [160].
- Regional species pool: The recovery of species that were locally exterminated from a disturbance such as pesticide pollution depends on, (1) the presence of these species in the regional species pool and, (2) that the ecological niche of this species is not occupied by a more competitive species [161].
- Climate: Several organisms develop faster and have more generations in regions with higher average temperature such as the tropics, which increases recovery of populations from disturbances [162, 163].
- Disturbance regime: the interaction with other disturbances and the type and frequency of other disturbances influence the recovery potential of the biotic community [128, 164].

To sum up, the recovery time of a freshwater community from pesticide stress is influenced by ecological, physicochemical, geographical and temporal factors.

How Frequent are Long-Term Effects Under Current Use Patterns?

In the previous sections we outlined the concentration levels that may cause long-term effects in the field. This raises the question, how frequently these exposure concentrations occur in the real world. For insecticides, Schulz [11] reviewed the concentrations given in field studies since 1982 and reported the maximum and minimum concentrations detected for each compound in each study. We calculated the respective $TU_{Daphnia}$ for the observed maximum concentrations for this data using the LC50 for *Daphnia magna* as given in the Pesticide Manual [165] in order to allow for a comparison with the long-term effect thresholds derived above. In the 64 studies, 162 of the 194 compounds measured, comprising 39 different insecticides, had concentrations above the limit of quantification. For these 162 observations, the $TU_{Daphnia}$ associated with the maximum concentrations exceeded 0.01 in 94 cases. Hence, in 58% of the observations in the respective field studies, the substances exhibited maximum concentrations that may cause long-term effects. To put this into the right context, one has to consider that: 1) some compounds without detections may not have been reported, hence the number of observations is limited to positive detections; 2) the study regions were not randomly selected but presumably based on some prior knowledge on pesticide pollution; 3) each of the 162 observations amalgamated up to 29 sampling sites and several sampling episodes; and 4) some regions with insecticide detections were sampled repeatedly [11]. However, a recent study on 83 pesticides in 17 agricultural streams over 4 years in the United States also reported that between 10% to 25% of the samples exceeded a $TU_{Daphnia}$ of 0.01 [166]. In addition, approximately 50% of the concentrations in the US streams exceeded a $TU_{primprod}$ of 0.01. By contrast, the TU_{fish} were rather low (most $TU_{fish} << 0.01$). Since no event-driven water sampler was employed in this study, the peak concentrations were most likely underestimated. Thus, the reported TUs represent a conservative estimate of the real exposure. Overall, the results confirm our conclusion that invertebrates and primary producers are at highest risk of being affected by pesticides and suggest that long-term effects of pesticides on both groups are not isolated cases.

RISK ASSESSMENT AND PREDICTION OF EFFECTS OF PESTICIDES

The beginning of widespread pesticide use in the middle of the 20th century was soon followed by reports of detrimental effects on ecosystems and human health [69]. Hence, today most countries require a pesticide risk

assessment for ecosystems and human health before authorisation of a substance is granted. The risk assessment procedure comprises a fate and an effects assessment. The fate side uses models and experimental data to assess the exposure in the environment (see chapter 2 in this book). In this chapter, we focus on methods to assess the effects; *i.e.* we give an overview of the different methods used to predict effects on aquatic ecosystems and describe their advantages and disadvantages.

Earlier, we mentioned that every effect of a pesticide has a physiological basis. However, the current science of ecotoxicology is very distant from a "grand unifying theory" that would mechanistically integrate all levels of biological organisation (Fig. **1**) and allows for the prediction of effects on the top levels from the lower levels. This holds true especially for the linking of suborganismal effects to higher levels. Currently, a clear link between responses at the suborganismal level and the fitness of individuals is still missing but would be a prerequisite for a sound suborganismal endpoint to be considered in risk assessment [167, 168]. Hence, although appealing from a precautionary principle point of view, suborganismal effects are at present no valid basis to predict effects on populations, communities or ecosystems. Currently, the approaches for ecological risk assessment of pesticides rely predominantly (1) on the individual (single-species laboratory tests) and ecosystem level and (2) on experiments. In the following we will sketch these as well as some alternative approaches.

Methods Relying on Toxicity on the Individual Level

The individual level has been the starting point of ecotoxicological research and still represents an important backbone supporting research on other levels of organisation. In fact, the vast majority of ecotoxicological data that has been produced to date (*i.e.* EC50 and LC50 data), originates from single-species toxicity tests. These tests allow for high replication and deliver relatively precise estimates of the toxicity endpoints (e.g. mortality or growth) under standardised conditions (temperature, water quality, age of test organisms *etc.*) [169]. For the first tier in pesticide risk assessment in a regulatory context, the acceptable concentration for a compound in the environment is derived by dividing the LC50 by a safety factor to account for uncertainties in the extrapolation from a single species in the laboratory to communities in the field. The uncertainties arise from abiotic and biotic factors that are not considered in single-species laboratory tests but may significantly modify the susceptibility of populations in the field such as ecological relationships within ecosystems [35, 170], recovery processes [36] and multiple stressors [32, 171]. In the European Union, a safety factor of 100 for acute toxicity tests for the invertebrate *Daphnia magna* and fish is applied to account for the above mentioned uncertainties [172]. The results from algal growth inhibition tests and chronic toxicity tests are divided by a safety factor of 10 to obtain the threshold concentration that should not be exceeded for the first tier in pesticide risk assessment. However, the use of this approach to predict effects in the field is relatively inefficient as it can be over- or underprotective [173, 174]. Underprotection of freshwater ecosystems can lead to losses of species and ecosystem services while overprotection may put unnecessary constraints on economic activities. Nevertheless, the single-species test is far less labour- and time-consuming than experiments on higher levels of biological organisation and are, therefore, an indispensable tool in the first tier risk assessment. Moreover, the results from these tests are critical for other research areas such as ecotoxicological modelling [175, 176], trait-based risk assessment [42, 94] or assessment of mixture toxicity [144, 177].

Alternative approaches to current single-species tests on the individual level can be categorised into: 1) those which use different methods to generate identical endpoints; and 2) those which generate different endpoints to assess the risk. Approaches of the first category include the use of different species or assays for testing and computational methods to predict toxicity data. Especially in the case of vertebrate testing, ethical concerns have promoted the development of alternatives such as the fish embryo test [178] or more recently the cell line test [179]. Another experimental development represents the rapid tolerance test that is a response to the scarcity of toxicity data for many compounds and species [113, 180]. Rapid testing involves simultaneous toxicity testing with field-collected taxa and sacrifices some precision in the determination of the toxicity endpoint in order to generate toxicity data on a wide array of species representative of natural communities [180].

A non-experimental method to obtain toxicity data is modelling. Quantitative structure activity relationship (QSAR) models represent a promising method to predict acute or chronic toxicity data [181, 182]. Here, the structure of compounds with known toxicity is used to predict the toxicity of unknown compounds. So far, QSARs have most successfully been applied to differentiate between narcotic and excess toxicity [183]. However, their value is

currently tenuous for compounds where the activity relies on exotic or unknown functional chemical groups. Moreover, several ecological models examine the influence of different test conditions on the determination of acute or chronic toxicity to allow for the adjustment to the respective field conditions [184]. For example, Ashauer *et al.* successfully (77% to 96% of explained variance) incorporated fluctuating and episodic concentrations of a pesticide using a threshold damage model to predict effects of realistic exposure conditions on the invertebrate *Gammarus pulex* [185], whereas standard toxicity tests utilise a constant or pulsed exposure. Finally, individual-level models can be used to analyse results from acute toxicity tests and explore mechanisms. For example, the energy budget model describes characteristics of individuals such as growth, metabolism or reproduction in terms of energy budgets [186] and the most commonly used energy budget model in ecotoxicology is DEBtox (see: http://www.bio.vu.nl/thb/deb/deblab/debtox/). However, most ecological models require toxicodynamic and toxicokinetic data that is available for a few species only and are therefore not widely applicable.

Species-sensitivity distributions (SSDs) present an alternative approach of the second category (generation of different endpoints) that was introduced to generate more accurate environmental quality targets [187]. SSDs integrate the results of single-species tests for a respective compound (or a mixture of compounds) to establish a statistical distribution of the sensitivity. The distribution function links the fraction of potentially affected species to the concentration of a compound. This allows for the derivation of a threshold concentration that is assumed to protect a defined percentage of taxa in the community (usually 95%). SSDs have received attention by regulators and are now used for the setting of environmental threshold concentrations in several countries including the US [188], the Netherlands, Australia and New Zealand [189, 190]. For herbicides and insecticides, two studies compared the thresholds derived with SSDs to effect concentrations observed in artificial ecosystem studies [99, 107]. For herbicides, SSDs based on chronic no-effect concentrations (NOEC) delivered threshold concentrations that were protective for artificial ecosystems, except for one out of nine compounds [99]. Similarly, the threshold concentrations derived from SSDs for 16 insecticides were protective for artificial ecosystems, though not in cases with repeated insecticide exposure where a safety factor of at least 5 was suggested [107]. However, species in artificial ecosystems are often more tolerant than natural communities [146] and therefore it remains to be demonstrated that SSDs are also protective in the field. Furthermore, the accuracy of SSDs for the prediction of effects in natural ecosystems has been questioned because they typically rely on the results of a few test species that are often not representative of natural communities and like single-species tests do not incorporate ecological relationships, multiple stressors or recovery processes [191]. In addition, their application range is limited due the scarcity of available toxicity data for many compounds [180]. In fact, toxicity data are often restricted to a few test species [135], while a minimum of 15 to 55 taxa have to be included in SSDs to arrive at thresholds with acceptable confidence limits [192], though as few as six taxa can be sufficient to derive protective concentrations [99]. The aforementioned rapid tests have been advocated as an experimental solution for the lack of species data [180]. Similarly, the use of, 1) expert judgement regarding the sensitivity of higher taxonomic groups (e.g., orders) combined with Bayesian statistical methods [193], and 2) statistical techniques such as interspecies correlation models [194, 195], allow for the construction of SSDs despite sparse data. Overall, SSDs represent a powerful tool to extrapolate individual level toxicity date to the community level, but do not consider ecological relationships so that the accuracy of the prediction is contentious.

Methods Relying on Toxicity on the Population level

Currently, impacts at the population level do not receive a lot attention in regulatory pesticide risk assessment [169]. However, since risk assessment is most interested in predicting effects on populations or communities, which are constituted of populations of many species, several authors have suggested that population level endpoints should inform risk assessment [196, 197]. Population level experiments can include ecological factors such as recovery, intraspecific competition and different life stages, and studies demonstrated that single species acute toxicity tests are a poor predictor for effects at this level [36, 198, 199]. The experiments can either be conducted for several populations in a community context such as artificial ecosystems that are discussed in the next section or for a single population. Yet, no consensus has been established on the experimental conditions in single population toxicity testing. More importantly, single population experiments are more labour and time-consuming and exhibit higher variability compared to single species tests [169], while not including ecological inter-species relationships and indirect effects. Therefore, advocates have focused on population level mathematical modelling for the prediction of effects [196]. The models can be broadly categorised into demographic models and individual-based models (for a

more thorough treatment of ecotoxicological population models see [196, 200]). Demographic models globally assign parameters such as fecundity or growth to age classes or the whole population and derive population level endpoints, which is most commonly the population growth rate. For example, in a study of the effects of diquat bromide on the bluegill (*Lepomis macrochirus*), the different age classes were parameterised with survival probabilities and fecundities and the model was used to assess the effects on the population growth rate [201]. Moreover, demographic models can be coupled with energy-budget models such as DEBtox to translate effects from the individual level to the population level [202]. By contrast, individual based models describe each individual in a population independently and the effects on the population level emerge out of the interaction and responses of the individuals when exposed to pesticides. These models can deliver new insights into mechanisms of toxic effects on populations and also allow for an explicit incorporation of the spatial dimension. For example, Van den Brink *et al.* [156] used an individual-based model to predict the effects of an insecticide on populations of the isopod crustacean *Asellus aquaticus* under different spatial scenarios. The results highlighted the relevance of habitat connectivity and the dispersal abilities, such as drift, of the species for its subsequent recovery in the impacted water body [156].

The major problem for ecotoxicological modelling on the population level remains the paucity of ecological data for other than the few better studied species, which hampers the parameterisation of models. This limits their value for prediction of pesticide effects. Nevertheless, they may be used to investigate effect mechanisms of pesticides in populations (and higher levels) to extrapolate empirical results from the individual level to the more meaningful population level. In addition, by modelling taxa based on generalised ecological traits such as generation time or dispersal capacity, population models can be valuable to select sensitive species or groups of species for the inclusion in artificial ecosystem experiments or identify indicator taxa in biomonitoring [149, 203].

Methods Relying on Toxicity on the Community and Ecosystem Level

The most frequently applied experimental method to predict effects of pesticides on the community level is ecotoxicological testing in replicated artificial ecosystems. Artificial ecosystem studies encompass different sizes and are accordingly differentiated into macrocosms, mesocoms and microcosms [204]. So far, most of the studies have been conducted in replicated mesocosms. Since mesocosms represent at least a part of an ecosystem, we do not draw a distinction here between the community level and the ecosystem level. In addition, mesocosm test systems possess all characteristics of real ecosystems such as ecological interactions, recovery processes, and depending on the experimental design, multiple stressors, though their configuration may be different.

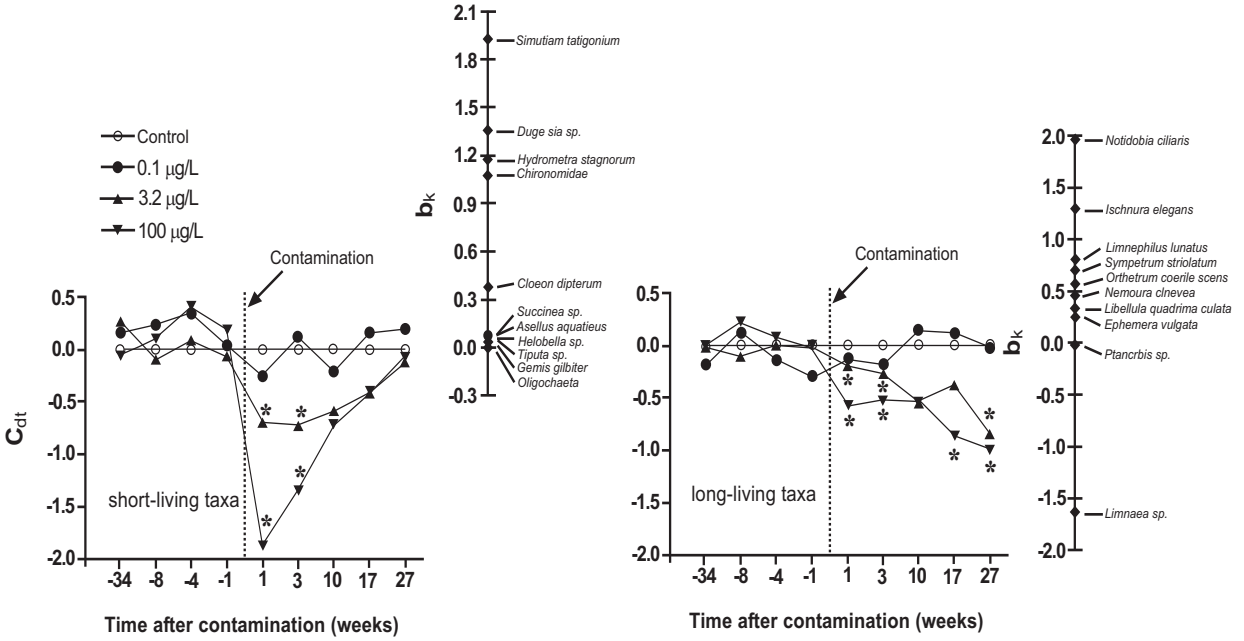

Figure 3: Effect duration of a neonicotinoid insecticide in mesocosms for short-living and long-living taxa. Asterisks indicate significant ($p < 0.05$, ANOVA, confirmed by both Games–Howell and Tamhane post-hoc tests) differences from the controls. C_{dt} = canonical coefficient for treatment d and week t. Modified and reprinted from [146] with permission from Elsevier.

Mesocosms can be constructed for both lentic (ponds) and lotic (stream) freshwater ecosystems. The construction of mesocosms starts with the physical containment structure, which is subsequently furnished with substrate and water. After allowing some time for stabilisation, sediments, plants and animals from natural ecosystems are introduced [204, 205]. The freshwater community should be established and replicate mesocosms should be similar in community composition before an experiment is run. Mesocoms can be constructed indoors or outdoors, with outdoor mesocosms being more realistic with exposure to field environmental conditions (rain, sun) but at the same time subject to higher variability due to seasonal variation and the risk of freezing during winter in temperate regions.

In general, experiments in mesocosm systems represent close-to-field conditions and include most biotic and abiotic factors that can influence the effects of a pesticide. Mesocosms have the advantage over field monitoring in that many factors can be controlled; the timing, duration and concentration(s) of exposure can be manipulated and the statistical power is higher since the abiotic factors between the replicated units are similar. Results from mesocosm experiments can be regarded as relatively accurate to predict effects in the field (effect thresholds) compared to other experimental methods and are therefore used as the highest tier in pesticide risk assessment. Nevertheless, there are some reasons why caution is warranted when predicting effects in the field from mesocosm experiments:

- Mesocosm communities do not necessarily mimic their natural counterparts under field conditions. The important trophic levels of vertebrates such as fish are often not included in artificial ecosystem experiments and the community composition for other trophic levels may be different compared to natural communities. Beketov *et al.* [146] surmised that long-term mesocosm studies had only 5-25% of long-living invertebrate taxa (uni- and semivoltine, life-cycle > 1 year) compared to 45-80% in natural streams.. Effects of the neonicotinoid thiacloprid on long-living invertebrate taxa persisted in a mesocosm study, while complete recovery of the short-living taxa occurred [146] (Fig. 3). Hence, potential long-term field-level effects would be underestimated when long-living taxa are underrepresented in the mesocosm system.
- Most studies were conducted in lentic systems and lentic communities contain different taxa and a lower fraction of sensitive taxa than lotic communities [206]. For example, 90 of 108 studies that were included in two meta-analyses on the effects of herbicides and insecticides were lentic [65, 108]. A lower fraction of sensitive taxa in the test system can lead to the underestimation of effects.
- Artificial systems are relatively variable over time because they are species-poor and lack redundancy [207]. At the same time they are poorly replicated and this raises concerns about the accuracy of the prediction. For example, mesocosm studies with esfenvalerate reported contradicting results concerning the development of primary production [208, 209], probably due to different ecological interactions between phytoplankton, zooplankton and fishes.
- Mesocosms have a low habitat complexity and only include a limited array of the various environmental conditions present in the field and therefore effects may still be over- or underestimated [196].
- It is uncertain to what extent the recovery dynamics of mesocosms are representative for the field situation. While in-stream recolonisation by drift from refugia or other, uncontaminated, sections of the freshwater system (mainly in lentic systems) is underestimated, external aerial recolonisation from other water bodies in the region may be stronger than in the field due to the presence of control streams in close vicinity.

The derivation of general concentration-response relationships that could be used in a predictive manner from field studies in freshwater ecosystems is difficult. This is because most field studies do not cover an exposure gradient as they are limited to a few streams or ponds (sample size < 10) and/or were not designed to deliver a regression. In addition, the causality between pesticide exposure and effects is often not clear [11]. In fact, field investigations on the effects of pesticides face two severe problems that hamper the establishment of a confident concentration-response relationship [42]. Firstly, the natural variation between communities at field sites is high, whereas reference sites often differ from pesticide-disturbed sites by more factors than only pesticide exposure [43]. Secondly, pesticide input during runoff events is associated with co-occurring changes in other environmental variables such as an increase in current velocity resulting in hydrological stress or increased turbidity that may confound effects of pesticides. We will outline three approaches to tackle natural variation and/or confounding factors. The first approach is to experimentally test the influence of potential confounding factors. Liess and Schulz

[210] constructed a bypass microcosm system connected to an agricultural stream and compared the effects of runoff with and without pesticide contamination on the dominant stream invertebrate populations. Only runoff events containing insecticides caused a significant decrease in the invertebrate populations [210]. In another study, the observed concentration range of environmental factors such as pesticide exposure and turbidity was investigated for effects on test species in laboratory experiments [211]. Since the test species showed only significant acute effects in response to pesticide contamination, the authors concluded that pesticides were the main cause of observed effects on invertebrate assemblages in the field [211, 212]. However, this approach is still confronted with natural variation when used to derive a concentration-response relationship.

A second approach to deal with natural variation and confounding factors represents the usage of ecological traits such as generation time or dispersal capacity to identify effects of pesticides [43]. The underlying ecological theory is that the sensitivity of taxa to a stressor and the occurrence of subsequent recovery patterns and indirect effects is dependent on their configuration of traits [161]. For example, species with a stream-lined body are more tolerant to hydrodynamic stress [213]. Hence, stressors can be regarded as a filter that selects taxa with a suitable trait configuration which results in an increase of these traits in the community [161]. Liess and von der Ohe [43] hypothesised that invertebrate taxa with a long generation time, low dispersal capacity, presence in water bodies during time of pesticide application and high physiological sensitivity would be most susceptible to pesticides and predicted a decrease of these "species at risk" (SPEAR) in the communities (see http://www.systemecology.eu/SPEAR/Start.html for online SPEAR calculator). In fact, they demonstrated a decrease of the fraction of sensitive taxa during the time of pesticide application in 20 streams in North Germany. A similar study was conducted in two regions of Finland and France and found a reduction in sensitive taxa with an increase in pesticide toxicity measured in terms of $TU_{Daphnia}$ [44]. Finally, an analysis of monitoring data comprising 28 tributaries in a Spanish river basin confirmed the relationship between pesticide input and decrease of sensitive taxa [135]. All studies established a significant relationship between pesticide toxicity and response of the sensitive taxa in the communities, or more technically speaking, between $TU_{Daphnia}$ and percentage of SPEAR species. Interestingly, the three concentration-response relationships were not significantly different and allowed for the derivation of an approximate effect threshold [42-44, 135]. According to these studies, slight effects occur already above a $TU_{Daphnia}$ of 0.001 and strong effects prevail above a $TU_{Daphnia}$ of 0.01 [44, 135]. This effect threshold lies approximately a factor of 10 below the effect threshold derived from mesocosm studies. The differences may be due to one of the following explanations:

1. The measured pesticide concentration (expressed as TU) in the field underestimates the peak exposure to which the ecosystem is exposed, resulting in a too low effect threshold.
2. The differences are due to different endpoints: the threshold for mesocosms refers to long-term changes in the communities, whereas the effect duration in the field studies is uncertain and the threshold may partly refer to observations of short-term effects.
3. The predictions from mesocosm studies underestimate the effects in the field due to differences in the factors outlined in the previous section (smaller fraction of sensitive taxa, different environmental conditions *etc.*)

Currently there is no consensus as to which explanation is most plausible. While some scientists argue that pesticide concentrations are usually underestimated in field monitoring [82] and consider the first explanation as the most likely, others emphasise the short-comings of mesocosm studies, which we have discussed earlier, and advocate explanation 2 or 3. However, if explanation 1 were true, this would mean that the different sampling methods (event-driven, grab sampling, passive sampling) employed in the three field studies (in some studies even in parallel) [22, 43, 44, 135] are subject to the same systematic error. More field studies would be needed to scrutinise this issue. Overall, the results from the abovementioned studies represent at least an accurate prediction of the order of magnitude at which effects may occur in the field. Nevertheless, the concentration-response relationship should be interpreted with care, when applying to, 1) areas with a different spectrum of applied pesticides, 2) lotic ecosystems, and 3) larger freshwater systems. In addition, the results can not be extrapolated to organisms other than invertebrates in the freshwater biotic community.

A third approach to tackle natural variation and confounding factors is the use of field-based sensitivity estimates. One method is similar to the SSD approach described before but uses sensitivity estimates from large field data sets to

predict thresholds. For example, a Norwegian dataset with 4200 sampling sites was used to assess the individual sensitivity of the frequently occurring and abundant taxa to toxicants and subsequently construct a field species sensitivity distribution (f-SSD)[214]. As with SSDs, the f-SSDs can be used to predict thresholds that should protect a certain fraction of the taxa in the community [214]. Recently, a new method was proposed by Kefford *et al.* [215] using dissimilarity indices to assess changes in the species pool across a contamination gradient. The method was applied to a larger data set on invertebrate data for 360 streams in North Germany and found a significant change in species composition with increasing modelled pesticide exposure [215, 216]. These methods are presumably more accurate in the prediction of effect thresholds than conventional SSDs relying on single species toxicity data. However, to apply this approach, large field monitoring data sets with concurrent pesticide measurements and biomonitoring data are required, which are very rare and presumably only available for governmental monitoring programs [135]. To date, governmental pesticide monitoring programs relied mainly on point water samples and were not adapted to detect episodic events such as pesticide runoff [82]. Hence, the predictions derived from these methods are likely less accurate than those from field studies using event-driven water samplers (see first section of this chapter).

Ecosystem Modelling

While many mechanistic models have been developed to predict the fate of contaminants in the environment, only a few mechanistic models target the effects of toxicants in freshwater ecosystems. These mechanistic effect models have only rarely been applied in ecotoxicology and are not thoroughly validated and compared to field data [217]. In one of the few published studies, Sourisseau *et al.* [217] calibrated and validated the ecosystem model Aquatox (http://www.epa.gov/waterscience/models/aquatox/) for control streams in a mesocosm experiment. The model was very sensitive to temperature parameters such as the optimal growth temperature for periphyton, filamentous algae and predatory invertebrates. Similarly, the optimal temperature for fish was a very sensitive parameter in another application of this model on the bioaccumulation of polychlorinated biphenyls [218]. Overall, these mechanistic models do not currently represent an alternative to other methods of prediction of effects and given the lack of ecological data for many species it is questionable if an adequate model can be used for the prediction of effects in the near future.

An alternative approach to mechanistic effect models is represented by statistical models that extrapolate observed concentration-response relationships from lower levels of biological organisation or from case studies to a larger scale. For example, Schriever *et al.* [219] combined a mechanistic fate model with a statistical model incorporating the concentration-response relationship observed in two central European regions to predict effects of pesticides on the European level [220]. However, due to data limitations and simplifications to allow for a wider prediction, such models do not provide accurate predictions of the effects, neither for specific pesticides nor on the small scale. Another example is the PERPEST model that predicts the magnitude and duration of effects of a certain concentration of a pesticide (and mixtures of pesticides) on various community endpoints simultaneously (e.g. community metabolism, phytoplankton and macro-invertebrates) [176, 221] and relies on a database containing results from freshwater mesocosm studies. A key advantage of PERPEST over single species/safety factor analyses is that it removes the need to extrapolate to the community level. However, the premise that the concentration-response relationships observed in a limited number of field case or mesocosm studies can be extrapolated to a wide range of freshwater ecosystems implicates uncertainties in the accuracy of the predictions. Nevertheless, due to the current limitations of mechanistic models and paucity of field studies, the statistical approach is certainly useful to identify potential hot spots of pesticide pollution and compounds of concern.

ACKNOWLEDGEMENTS

The authors like to thank Ben Kefford, Peter von der Ohe and three anonymous reviewers for valuable comments that helped to improve the quality of the manuscript. Special thanks to Mikhail Beketov for providing Fig. (3). RBS received financial support (SCHA 1580/1-1) from the German Science Foundation (DFG).

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